

P001

Photo-affinity Probes for Cell-Based Proteome Profiling of Potential 3-Deazaneplanocin A Targets

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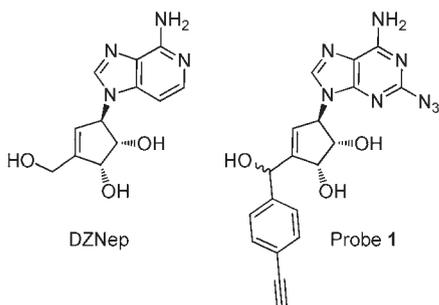
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Development of effective and safe drugs is the 'Holy Grail' of medicinal chemistry. For small-molecule drugs, which comprise most of today's medicines, a key challenge is the identification of molecular targets that lead to therapeutic effects (on-target) and/or adverse side effects (off-target). Recently, a histone methylation inhibitor, 3-deazaneplanocin A (DZNep),^[1] has attracted significant interest in epigenetic therapy.^[2] It is known to inhibit EZH2 complex and the associated H3K27 trimethylation, leading to apoptosis in cancer cells and in cancer stem cells but not in normal cells.^[3] However, the molecular mechanism of action is not well understood. Our aim is to use cell-based proteome profiling methods^[4] to gain some insights on the biological targets that may be involved. Through a cell-based screening study, we successfully identified probe **1**, which possesses similar antiapoptotic activity as compared with DZNep. This compound was specially designed to contain a 'photo-warhead' (binds irreversibly to the active site) and an 'alkyne handle' (by conjugation to a reporter tag via a Huisgen cycloaddition reaction). Details of the synthesis and biological results will be presented.



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P002

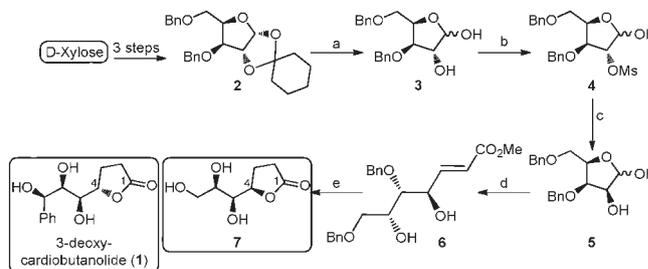
Synthesis and Antiproliferative Activity of a New 3-Deoxy-cardiobutanolide Analogue

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A total synthesis of new hydroxy lactone **7** was completed starting from D-xylose (Scheme 1). Compound **7** was designed as a dephenylated analogue of cytotoxic 3-deoxy-cardiobutanolide (**1**),^[1] with inverted configuration at C-4. Compound **2**, which was easily available from D-xylose, was converted to lactol **3** after hydrolytic removal of the cyclohexylidene protective group. Treatment of **3** with mesyl chloride gave 2-O-mesyl derivative **4**. Treatment of **4** with NaOH affected the C-2 epimerisation, whereby corresponding D-lyxo derivative **5** was obtained. Stereoselective Wittig olefination of **5** gave E-enoate **6**, which was finally converted to target **7** after catalytic reduction/hydrogenolysis, followed by acid-promoted lactonisation. Compound **7** was evaluated for its in vitro antiproliferative activity against selected human tumour cell lines.



Scheme 1. Reagents and conditions: a) 80% AcOH, reflux, 10 h; b) MsCl, abs. Et₃N, abs. CH₂Cl₂, -15°C, 2.5 h; c) 0.1 M aq NaOH, DMF, RT, 1.5 h; d) Ph₃P=CHCO₂Me, abs C₆H₆, reflux, 1.5 h; e) i. H₂, 10% Pd/C, MeOH, RT, 23 h; ii. TFA/H₂O (2:1), RT, 20 h.

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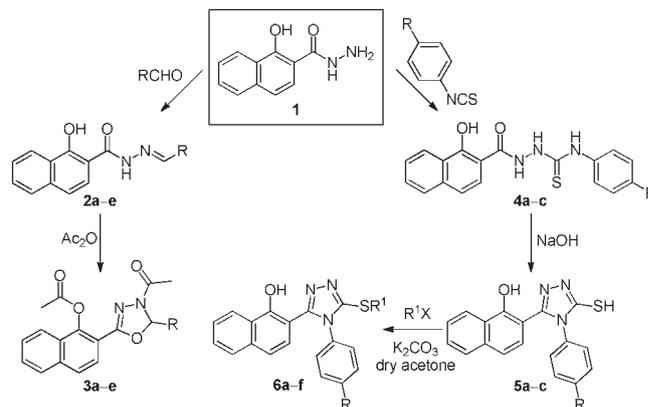
P003

Discovery and Synthesis of JAK2 Inhibitors

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JAK2 is a member of the Janus kinases (JAKs), which are intracellular nonreceptor protein tyrosine kinases including Jak1, Jak2, Jak3, and Tyk2. JAK2 phosphorylates specific tyrosine and serine residues of the JAK2 receptor, creating docking sites for the signal transducers and activators of transcription (STATs). Then, STATs bind the receptor, allowing JAK2 in turn to phosphorylate STATs. Finally, phosphorylated STATs are dissociated from the receptor, form dimers, and translocate into the nucleus, where gene transcription is regulated. Ninety-one structurally diverse compounds containing nicotinamides, bis-amides and quinazolines were tested for JAK2 inhibition. The most potent compounds, WJ042 and WJ023, were further investigated for inhibitory effects on STAT3 phosphorylation and target gene expression, and they strongly reduced JAK2 activation, subsequent STAT3 phosphorylation, and antiapoptotic protein levels. Intriguingly, these compounds showed strong cytotoxicity in a dose-dependent manner. This good correlation between JAK2/STAT3 inhibition and cytotoxic effect of the compounds demonstrates the nicotinamides and bis-amides would be potential leads for developing inhibitors of JAK2/STAT3 signaling pathway as antitumor agents.



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P004

Synthesis and Biological Evaluation of Novel Phenolic Derivatives as Potential Anticancer Agents

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Phenolic derivatives represent an important class of anticancer agents.^[1–3] In addition, oxadiazoles^[4–7] and triazoles^[8–11] are known to possess anticancer activity. These facts encouraged us to synthesize new α -naphthol derivatives linked to oxadiazoles and triazoles hoping to yield highly active antitumor agents. Some of the prepared compounds were selected by the National Cancer Institute (NCI), Bethesda, Maryland, USA to be screened for anticancer activity against 60 different tumor cell lines. The following scheme illustrates the preparation of target compounds.

P005

Galloylation of Flavonolignans Improves Their Antiangiogenic Activities

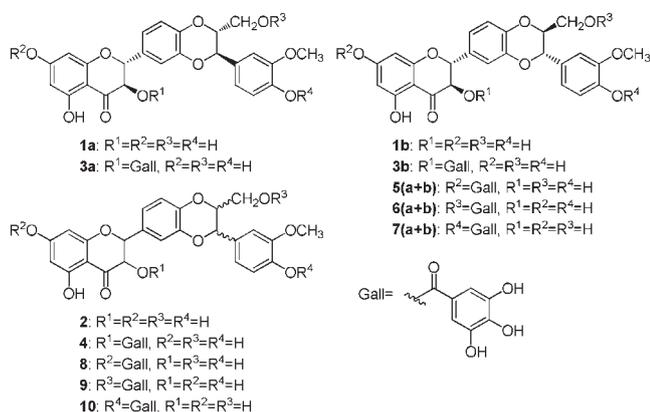
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Flavonolignans silybin (**1**) and 2,3-dehydrosilybin (**2**), isolated from milk thistle (*Silybum marianum*), are well-known flavonoids with broad spectra of biological activities operating at various cell levels.^[1] Silybin is mainly used in the prevention and treatment of various liver diseases and as a protectant against a number of hepatotoxins and mycotoxins. Moreover, both silybin and 2,3-dehydrosilybin have been identified as rather effective natural compounds in the prevention and treatment of some types of cancer (e.g., prostate cancer). One of the mechanisms of silybin anticancer activity consists in its antiangiogenic effects—a complex process involving several particu-

lar events.^[2] The presence of galloyl moiety in the structure of flavonoids (e.g., catechins) was found as another important prerequisite for their significant antiangiogenic properties (typically in EGCG).^[3]

The synthesis of various silybin and 2,3-dehydrosilybin mono-galloyl esters was developed and their antiangiogenic activities were evaluated in a variety of in vitro tests with human umbilical vein endothelial cells (HUVECs). Moreover, the regioselectivity of the silybin galloylation was shown to be highly significant for resulting activity in our structure–activity relationship study. The most effective compound from silybin series—7-*O*-galloylsilybin (**3**)—has also been prepared from stereochemically pure silybin A and B to evaluate the effect of stereochemistry on the activity. As with silybin itself, the **3b** isomer was more active than the **3a** isomer. Moreover, preliminary antiangiogenic tests of 2,3-dehydrosilybin galloyl-esters show that these compounds possess even better activities than the corresponding silybin gallates.



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P006

Discovery and Development of a Potent and Orally Bioavailable HCV NS5A Inhibitor DBPR110

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Hepatitis C virus (HCV) is the major cause of liver disease worldwide and a potential source of high morbidity and mortality in the future. According to the World Health Organization, approximately 180 mil-

lion people, 3% of the world's population, are chronically infected with HCV, and 3–4 million new infections occur each year. Thus there is an urgent need for the development of more efficacious and better tolerated anti-HCV agents. The HCV non-structural 5A protein (NS5A) has generated wide interest in HCV research because of its key roles in both viral RNA replication and modulation of cellular pathways and processes, including innate immunity and host cell growth and proliferation. Recently, we reported the synthesis and identification of DBPR110, a potent and selective small molecule inhibitor against NS5A protein. DBPR110 was synthesized in seven steps and exerted excellent anti-HCV activity in a 1b replicon assay (EC₅₀=3 pm). In addition, it also showed good pharmacokinetic properties with desired oral bioavailability in both rats and dogs. Therefore, DBPR110 represents a promising candidate for potential use in the treatment of HCV infection.

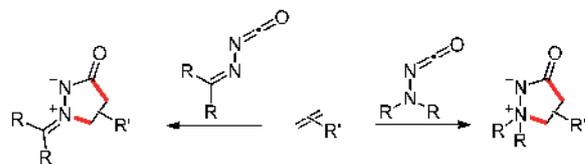
P007

A Simple Approach to β -Aminocarbonyl Motifs using Amino- and Imino-Isocyanates

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Over the past recent years, β -aminocarbonyls have been of great interest to medicinal chemists. As a practical method to obtain these moieties, alkene aminocarbonylation, accounting for the formation of a C–N and a C–C bond, has been the subject of few research efforts (only specific intramolecular metal-catalyzed variants have been reported). Direct aminocarbonylation of alkenes constitutes a challenge and an important potential innovation in the synthesis of β -aminocarbonyls such as β -amino acids. Recently, efforts from our group have been directed towards the development of concerted pathways for the amination of alkenes. Building on our previous report on the reactivity of hydrazides,^[1] recent progress on the intra- and intermolecular aminocarbonylation of alkenes with amino- and imino-isocyanates along with the synthetic scope of this reactivity will be discussed. In addition, a practical and efficient synthesis of diverse β -aminocarbonyls will be presented.



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P008

Evaluation of Anticancer Activity of Indolylacrylamide Derivatives

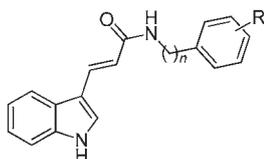
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Cancer is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Chemotherapy is a major form of treatment for cancers. Nevertheless, from early on in the development of chemotherapy, it was realized that the window in which the dose range is both efficacious and safe is small. Consequently, the principal obstacles to the clinical efficacy of chemotherapy remain their possible toxicity to normal tissues of the body. Moreover, the majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. As a result, the design and discovery of nontraditional, efficient and safe chemical classes of agents are prime targets in contemporary medicinal chemistry.

Chalcones are structurally similar to indol derivatives having heterocyclic unit at three position. Indol-based chalcones are explored for their anticancer potential. In our effort to discover and develop potential new anticancer agents, we synthesized a series of novel indolylacrylamide derivatives which are similar to indolyl chalcone structure and evaluated for their anticancer activity.



P009

Synthesis of Conformationally Restricted κ Receptor Agonists by Double Henry Reaction

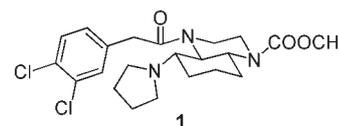
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The κ -opioid receptor is one of three opioid receptor subtypes (μ -, κ - and δ -receptor), and its activation leads to strong analgesia.^[1] Our attention has been focused on the development of selective κ agonists as potent analgesics to avoid undesired side effects like respiratory depression or physical and psychological dependency. The undesired side effects of κ agonists (e.g., hallucination, dysphoria) could be prevented by developing polar κ receptor agonists, which cannot pass the blood–brain barrier.

In the last years, studies showed that the κ affinity of arylacetamide agonists strongly depends on the torsion angle of its ethylenediamine substructure.^[2] Based on this information, it would be interesting to synthesize new, conformationally restricted κ agonists to investigate the bioactive conformation.

In our group, different bridged, conformationally restricted compounds with piperazine structure have been developed. The very potent and selective compound **1** has a K_i value of 9.7 nM.^[3] To improve the κ affinity, it would be interesting to synthesize analogues of **1** with a cyclopentan or indane moiety instead of the cyclohexane ring. Herein, the optimization of the double Henry reaction with 1,4-dialdehydes is described, which represents the first key step in the synthesis.



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P010

Inhibition of Protein–Protein Interactions using Designed Molecules

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Protein–protein interactions (PPIs) play a pivotal role in diseased states and so there is a pressing need for synthetic agents that selectively target these interfaces.^[1] What is not clear is how to do this using a small molecule, given that it must cover 800–1100 Å² of a protein surface and complement the discontinuous projection of hydrophobic and charged domains over a flat or moderately convex surface.^[1] Several general approaches tailored to particular protein topologies are emerging for the design of scaffolds that inhibit PPIs including: proteomimetics and surface mimetics.^[1] Proteomimetics replicate the spatial projection of key binding residues from a secondary structural motif important in the target PPI whilst surface mimetics present recognition domains from a core scaffold in a multivalent manner to achieve high-affinity protein surface recognition. This presentation will outline our work in both areas (Figure 1).

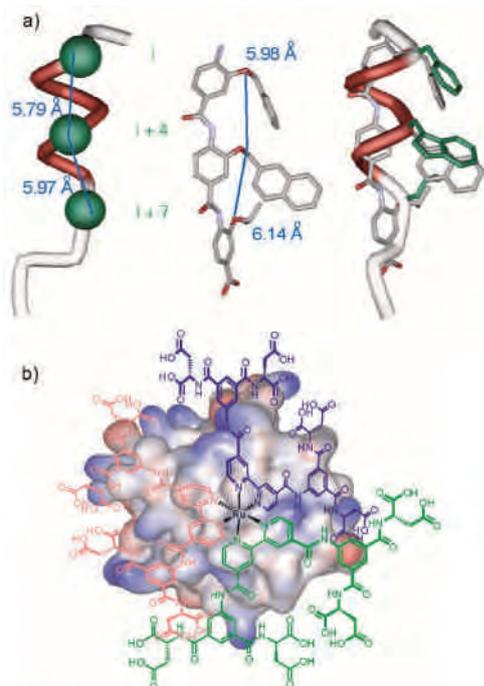


Figure 1. a) Aromatic oligoamide proteomimetics and b) protein surface mimetics based on ruthenium tris(bipyridine) complexes.

The development of solid-phase syntheses of aromatic oligoamides amenable to library generation will be described alongside screening results^[2,3] that illustrate such compounds act as μM inhibitors of the p53-hDM2 interaction.^[4] Screening against the Bcl-2 family of PPIs alongside further biophysical analysis will be presented. The design and synthesis of highly functionalised ruthenium tris(bipyridine) complexes that act as tuneable and cell-permeable nm affinity receptors for proteins such as cytochrome c will also be described.^[5,6]

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P011

Design and Synthesis of MMP-2 Inhibitor–Quantum Dot Conjugates

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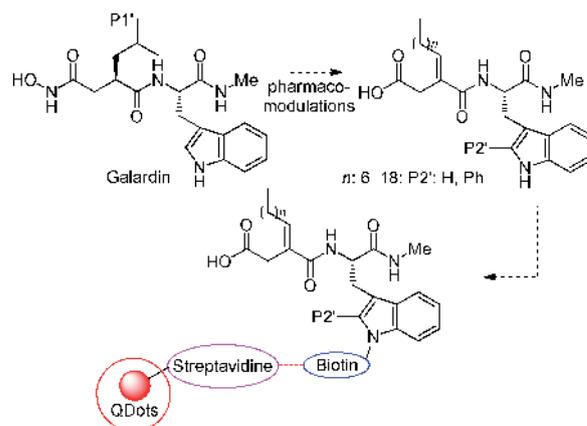
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Human melanoma accounts for less than 5% of skin cancers, but it is responsible for 80% of mortality, and its incidence has doubled worldwide in the past 20 years. Although several matrix metalloproteinases (MMP-1, MMP-2, MMP-14) have been found to favour melanoma cell invasion by their capacity of degrading collagen, several arguments pointed towards MMP-2 as a main collagenase target in melanoma either alone as overexpressed by cancer cells or fibroblasts or in combination with tumor-derived MT1-MMP. Consequently, MMP-2 is now considered as a main protease able to degrade mutant or modified collagen occurring in sun-exposed skin.^[1]

Quantum dots (QDs) have recently emerged as valuable tools in bioanalysis, biological imaging and studying complex biochemical interactions. QD-MMP inhibitor conjugates may generate very sensitive new tools displaying a dual role, i.e. detection of MMPs expression and inhibition of MMPs activities enabling the evaluation and control of melanoma progression.^[2]

In continuation of our pharmacomodulation studies^[3] of Galardin®, a potent but nonselective MMP inhibitor, we describe herein the synthesis of new pseudodipeptide-type inhibitors coupled to QDs. The obtained inhibitors have been characterized by their inhibitory activity and specificity towards MMP-2. Biological results showed that introduction of a long alkyl chain ($n=8$) in P'1 position and a phenyl group on the indole C-2 carbon were beneficial to afford more potent and selective MMP-2 inhibitors. MMPI-QD conjugates were prepared by biotinylation followed by treatment with streptavidin-associated QDs.



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P012

Discovery and Optimization of Novel Purines as Potent and Selective CB2 Agonists

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Cannabinoid receptors CB1 and CB2 belong to the class of G-protein-coupled receptors (GPCRs). CB1 receptors are expressed both centrally and peripherally while CB2 receptors are predominately expressed peripherally, primarily on immune cells and tissues. The pharmacological and therapeutic potential of the CB2 receptor has been reviewed recently identifying CB2 as a therapeutic target for the treatment of pain, in particular, inflammatory and neuropathic pain.

This poster will describe the discovery of a series of Purine compounds that were found to be highly selective for CB2 receptors over CB1 therefore avoiding unwanted CNS side effects. The poster will focus on the optimization of the series to solve issues of high metabolism and cross reactivity in order to discover a clinical candidate, selective with no cross-reactivity, high solubility and active in models of OA pain.

P013

pH-Sensitive Steroidal Antiestrogen–Doxorubicin Conjugate for ER-Positive Breast Cancer Drug Delivery

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As part of our program to develop breast cancer specific therapeutic agents, we have synthesized a conjugate-agent that is a conjugate of the steroidal antiestrogen and the potent cytotoxin doxorubicin. In this effort, we employed a modular assembly approach to prepare a novel 11 β -substituted steroidal antiestrogen functionalized with an azido-tetraethylene glycol moiety which could be coupled to a complementary doxorubicin benzoyl hydrazone functionalized with

a propargyl tetraethylene glycol moiety. Huisgen [3+2] cycloaddition chemistry gave the final hybrid that was evaluated for selective uptake and cytotoxicity in ER(+)-MCF-7 and ER(-)-MDA-MB-231 breast cancer cell lines. The results demonstrated that the presence of the antiestrogenic component in the hybrid compound was critical for selectivity and cytotoxicity in ER(+)-MCF-7 human breast cancer cells as the hybrid was ~70-fold more potent than doxorubicin in inhibition of cell proliferation and promoting cell death.

P014

Design of Pyrido[3,2-d] and [2,3-d] Pyrimidines as Dual PI3K/mTOR Inhibitors

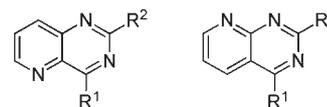
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Kinases catalyze cell reactions and are indispensable to the cellular mechanism such as cell proliferation, survival, growth and angiogenesis.^[1] According to various studies, it has been observed in cancer patients, activating mutations of enzyme genes that lead to elevated pathway activities.^[2] In order to cure cancer, research on combined therapies (radio and chemotherapy) was strongly developed within those last years as it has proven to be much more beneficial for the patient. We chose to inhibit the PI3K/Akt/mTOR pathway in particular, often mutated in various types of cancers, and thus is a promising target in therapeutic research.

Based on previous studies,^[6] we have decided to synthesize PI3K/mTOR inhibitors using a pyridopyrimidine scaffold. By developing efficient and adequate synthesis strategies, we have increased our products abilities to inhibit these enzymes. A structural optimization, based on molecular modeling studies, was elaborated using several synthetic pathways which will be described.

In parallel to the syntheses, we have tested and optimized four kinase assay kits. We have selected one of them with the criteria: easy to use, reproducible and reliable. We have tested our compounds and the SAR will also be presented.



R¹: Cycloalkyl amine; R²: Aromatic cycle

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P015

Part II: Synthesis, Cancer Chemopreventive Activity and Molecular Docking Study of Novel Quinoxaline Derivatives

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Cancer chemopreventive agents are designed to reduce the incidence of tumorigenesis by intervening at one or more stages of carcinogenesis. The cancer chemopreventive activity of quinoxaline derivatives **1–20** has been evaluated by studying their possible inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Quinoxaline derivatives **1–20** showed inhibitory effects on EBV-EA activation without cytotoxicity on Raji cells. All compounds exhibited dose-dependent inhibitory activities, most of them showed significant activity at 1000 mol ratio/TPA. Compounds **7** and **9** exhibited strong inhibitory effects on the EBV-EA activation, and their effects being stronger than that of a representative control, oleanolic acid, at the highest concentration used. Moreover, the molecular docking into PTK (PDB: 1t46) has been done for lead optimization of the aforementioned compounds as potential PTK inhibitors.

Keywords: synthesis; quinoxalines; Epstein–Barr virus; cancer chemopreventive activity; 12-*O*-tetradecanoylphorbol-13-acetate (TPA); docking; protein tyrosine kinase (PTK).

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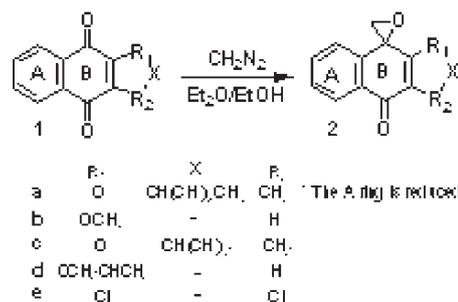
P016

New Oxyran Derivatives of 1,4-Naphthoquinones and their Evaluation against *Trypanosoma cruzi* Epimastigote Forms

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Introduction: Chagas disease is an endemic disease caused by *T. cruzi*. Approximately 16 to 18 million people are infected, and 50,000 individuals die each year from this disease.^[1] Only two drugs have been used since the 1970s: nifurtimox and benznidazole. Several synthetic substances continue to be reported in the literature,^[2a,b] but none have become a new drug for a treatment. An α -lapachone derivative has shown potent trypanocidal activity and low cytotoxicity.^[3a,b] The objective of this work was to synthesize new oxyran derivatives obtained from quinones and to evaluate them against *T. cruzi*.

Experimental: A solution of diazomethane in ethyl ether was added to a solution of naphthoquinone in diethyl ether/ethanol (3:1). The reaction was carried out at room temperature for 48–120 hours. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate as the eluent.



Scheme 1. General route for the preparation of oxyran derivatives from 1,4-naphthoquinones.

T. cruzi Y epimastigote forms were treated with 50 μ M of each compound for 72 hours. The cells were centrifuged and then incubated with 30 μ g/mL of propidium iodide for 15 minutes. Data were analyzed using a C6 flow cytometer. These cells were then incubated for 72 hours at 28 $^{\circ}$ C in BHI medium supplemented with 10% fetal bovine serum. Trypanocidal effects were quantitatively monitored by direct counting in a Neubauer chamber using optical microscopy.

Results and Discussion: Only compound **2a** showed a mortality rate lower than benznidazole (Table 1). All oxyran ring-containing compounds showed lower cytotoxicity than the naphthoquinones from which they were derived. Compound **2b** appears to be the best candidate for use as a trypanocidal agent. Benznidazole was used as a control with an IC₅₀ value of 11.5 mM and a CC₅₀ value of 40 μ M.

Table 1. IC₅₀ and CC₅₀ values of naphthoquinones and their respective oxyran derivatives.

Quinolone	IC ₅₀ [mM]	CC ₅₀ [μM]	Oxyran	IC ₅₀ [mM]	CC ₅₀ [μM]	Yield [%]
1a	16.33	11.7	2a	16.38	58.1	70
1b	3.19	13.02	2b	1.13	44	80
1c	8.8	2.7	2c	19.33	19	60
1d	0.02	<1	2d	0.2	<1	35
1e	0.09	6.3	2e	9.48	19	52

Conclusions: Oxyran derivatives exhibited reduced cytotoxicity in mammalian cells compared to their corresponding quinones. Compound **2b** showed high trypanocidal activity and low cytotoxicity, comparable to benzimidazole. Thus, compound **2b** emerges as a promising candidate for the development of a new drug for the treatment of this disease.

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P017

Synthesis and Antimycobacterial Properties of 5-Chloro-*N*-phenylpyrazine-2-carboxamides

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Although both relative and absolute incidence rates of tuberculosis (TB) have started to decrease slowly in recent years, TB remains a threatening infectious disease. The emerging resistance of pathogenic *Mycobacteria* to currently used drugs is the driving force for development of new antituberculars, preferably with novel mechanism of action.

5-Chloropyrazine-2-carboxamide (5-Cl-PZA) exerts “in vitro” activity against *Mycobacterium tuberculosis* mutants resistant to pyrazinamide as well as against mycobacteria naturally resistant to pyrazinamide (*M. bovis*, *M. kansasii*, *M. avium*, *M. fortuitum*, *M. smegmatis*).^[1] 5-Cl-PZA was showed to inhibit the mycobacterial fatty acid synthase I (FAS I).^[2] Therefore, we believe 5-Cl-PZA scaffold might be used to design new potent antituberculars with broad activity. Some anilides of pyrazinecarboxylic acid (with different substitution in both pyrazine and/or phenyl ring) already proved to be active.^[3]

Commercially available 5-hydroxypyrazine-2-carboxylic acid was used to synthesize the title compounds in a convenient two-step synthesis. More than 25 new anilides of 5-chloropyrazine-2-carboxylic

acid were screened for in vitro antimycobacterial activity (microdilution broth method) against *M. tuberculosis* H37Rv, *M. kansasii* and two stems of *M. avium*.

5-Chloro-*N*-(2,3-dichlorophenyl)pyrazine-2-carboxamide inhibited *M. tuberculosis* H37Rv with an MIC value of 3.13 μg/mL (approx. 10 μmol/L); the MIC value of PZA standard was 6.25–25 μg/mL (50–203 μmol/L). Other compounds were also active against strains resistant to PZA.

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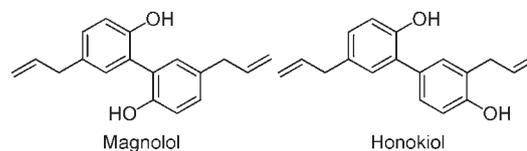
P018

New Biphenyl Diol Derivatives Display Multikinase Inhibitory Properties

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We present new magnolol and honokiol derivatives showing inhibitory activity against multiple protein kinases, including epidermal growth factor receptor (EGFR), proto-oncogene tyrosine-protein kinase Src, cyclin-dependent protein kinase (CDK2), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK 1). Antiproliferative activity (IC₅₀=0.9–4.6 μM) has been demonstrated in brain, colon, liver, ovary, prostate and breast tumor cell lines, as well as melanoma and leukemia cell lines. These novel compounds have potential to suppress tumor growth and/or prevent recurrence of metastasis. In vivo efficacy of the compounds has been proven using tumor xenografts models. Furthermore, analgesic activity of claimed compounds has been observed in experimental animal models of pain.



P019

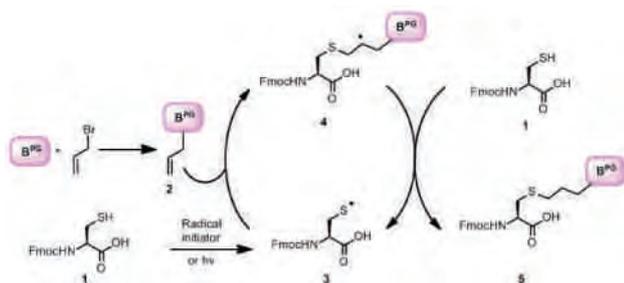
Synthesis of New Cysteine-Based Building Blocks of Alpha-Peptide Nucleic Acids (Alpha-PNAs) via Thiol-Ene Reactions

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Alpha-peptide nucleic acids (α -PNAs) are a class of functional analogues of the natural oligonucleotides where the ribose-phosphate backbone has been replaced by a true peptide made up of α -amino acids, some of them carrying a nucleobase in the side chain.^[1] α -PNAs bind to complementary RNA or DNA following the Watson-Crick base pairing rules preferentially in an antiparallel orientation.^[2] PNAs have several advantages over the natural nucleic acids, such as: (i) resistance to nucleases and proteases, (ii) exhibit little or no binding to serum proteins, (iii) higher affinity and sequence specificity to complementary nucleic acids than DNA/DNA duplexes, and (iv) higher chemical and thermal stability.^[3] In view of these properties, PNA technology has gained importance in several research fields, from molecular diagnostics to drug discovery.

In this work, we present the synthesis of new chimeric cysteine derivatives with nucleobase side chains that can be used as α -PNAs building blocks. To synthesize these molecules we used thiol-ene reaction in which a cysteine thiol radical (**3**) adds to a alkene double bond in the nucleobase linking chain (**2**) (previously Boc-protected and N-alkylated), producing a carbon radical (**4**), which in turn can abstract a new hydrogen from another cysteine thiol group (**1**) and thus propagating the cycle.^[4] Although the formation of the cysteine thiol radical from a cysteine molecule can be promoted by a radical initiator and by UV light, we observed that better yields are obtained when the photochemical approach is used.



Scheme 1. Synthesis of cysteine-based building blocks via thiol-ene reaction (B^{PG}: Boc-protected nucleobase).

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P020

Structure-Based Drug Design of Cytochrome bc₁ Complex Inhibitors Targeting the Q_i Binding Site of *Plasmodium falciparum*

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Malaria remains a serious global health problem, being one of the most lethal diseases in tropical countries. Unfortunately, it becomes hard to control due to the rapid and continuously emergence of drugs resistance.^[1,2] Having this in consideration, there's an urgent need for new drugs that preferentially act on underexploited parasite targets. Of the five parasite types, *Plasmodium falciparum* is the most virulent and is responsible for more than 95% of malaria-related morbidity and mortality.^[3] Several studies proved that blocking the electron transport chain in *plasmodium* compromises the cellular vital functions promoting cells death.^[4,5]

A virtual screening was performed targeting the Q_i pocket of the bc₁ complex of *P. falciparum*.^[6] Since the crystallographic structure of the bc₁ complex of *P. falciparum* is not available, a homology model of the parasite's cytochrome *b* was obtained and validated, and several databases were screened by a docking protocol against this particular Q_i site. After a carefully selection, the highest scored compounds were purchased, and the evaluation of their biological activity against bc₁ complex is also object of our study.

Acknowledgements: The authors acknowledge FCT for funding the project PTDC/SAU-FCF/098734/2008.

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P021

New Solutions for an Old Problem: Surpassing the Pharmacokinetic Drawbacks of Natural Phenolic Antioxidants

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The development of new antioxidant entities is an increasingly important research area in the field of medicinal chemistry. Oxidative damage induced by reactive oxygen and nitrogen species (ROS and RNS, respectively) is inherent to inflammation processes, which in turn play a key role on a wide range of pathologies, from cancer to neurodegenerative diseases (ND).^[1]

Phenolic acids are naturally occurring compounds that exhibit potent antioxidant activity by different mechanisms such as scavenging ROS and RNS, binding to pro-oxidant transition metals (mainly Cu and Fe) and inhibiting ROS/RNS-generating enzymatic systems.^[2] The combination of these mechanisms hinders both the initiation and progression of free radical formation blocking or minimizing the oxidative damage cascade. Furthermore, epidemiological studies suggest an inverse relationship between dietary intake of phenolic antioxidants and the occurrence of diseases such as cancer and ND.^[3]

Hydroxycinnamic acids are ubiquitous phenolic compounds, accounting for approximately one third of the phenolic compounds in our diet. To date, the majority of natural antioxidants studied have limited therapeutic success a fact that could be related with their limited distribution throughout the body and with the inherent difficulties to attain the target sites. So, if conditions are met to overpass the mentioned drawbacks these compounds can efficiently operate as potent exogenous antioxidants and in that way supplement the body's endogenous antioxidant defence systems.

As antioxidant activity is known to be strongly dependent on the compound's structural characteristics,^[4] a project was designed related to the development of novel cinnamic acid derivatives aiming an increase in lipophilicity and, subsequently, the efficacy of the natural compound. The overall structural modifications would enable a better diffusion across the membrane and, ultimately, better antioxidant activity. The results obtained so far will be presented in this communication.

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P022

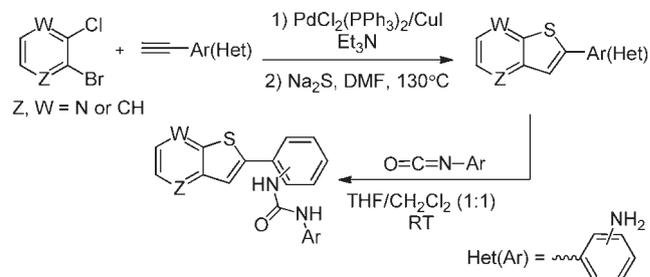
Synthesis of Thienopyridine Derivatives as Potential Antitumorals and/or Antiangiogenics

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Thienopyridine derivatives have been shown to exhibit a large variety of biological activities, thus attracting considerable attention. For some years now, our research group has been interested in the synthesis of thieno[3,2-*b*]pyridines susceptible to present antitumoral^[1-3] and antiangiogenic activities.

Herein, we present a new methodology for the synthesis of thieno[3,2-*b*]pyridines and thieno[2,3-*b*]pyridines bearing various (hetero)aryl substituents in the 2-position, from 2,3-dihalopyridines and (hetero)arylalkynes through a Sonogashira coupling followed by reaction with Na₂S and intramolecular cyclization (see scheme). The synthesized thienopyridines bearing an aniline in position 2 were reacted with arylisocyanates to give 1,3-diarylureas in the thienopyridine series.



The latter could act as tyrosine kinase inhibitors of vascular endothelium growth factor receptor 2 (VEGFR2), a key component of the signaling pathway responsible for the sprouting and maturation of new blood vessels from tumors, as various thieno[3,2-*b*]pyridine ureas have already been shown to be potent inhibitors of VEGFR-2.^[4]

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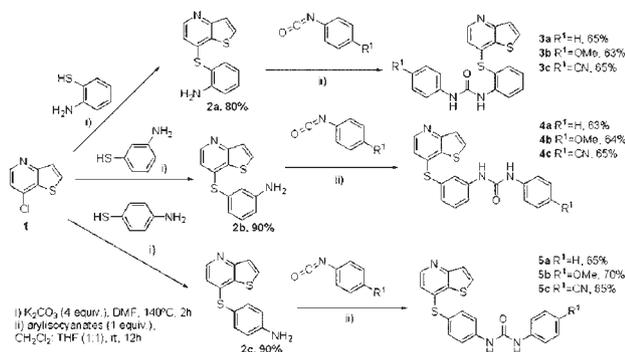
P023

Synthesis of Novel 1-Aryl-3-[2-,3- or 4-(thieno[3,2-*b*]pyridin-7-ylthio)phenyl]ureas and Evaluation as VEGFR2 Tyrosine Kinase Inhibitors

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Vascular endothelial growth factor receptor 2 (VEGFR2) tyrosine kinase is involved in cancer and in angiogenesis.^[1] Herein, we report the synthesis of novel 1-aryl-3-[2-, 3- or 4-(thieno[3,2-*b*]pyridin-7-ylthio)phenyl]ureas as VEGFR2 inhibitors by promoting the regioselective attack of the thiol group of the 4-aminothiophenol in the chlorine nucleophilic displacement on 7-chloro-2-thienopyridin-5-ylthio-4-aminophenol **1**, obtaining the aminated compounds **2a–c**. These were reacted with arylisocyanates to give the corresponding 1,3-diaryliureas **3a–c**, **4a–c** and **5a–c** (see scheme).

1-Aryl-3-[3-(thieno[3,2-*b*]pyridin-7-ylthio)phenyl]ureas **4a–c** with the arylurea in the *meta* position relative to the thioether showed the lowest IC₅₀ values (0.4–0.9 μM) in enzymatic assays using VEGFR2 tyrosine kinase domain, and the binding mode for these compounds was predicted by docking simulations.

Acknowledgements: The Foundation for Science and Technology (FCT–Portugal) is acknowledged for financial support through the NMR Portuguese network (Bruker 400 Avance III–Univ Minho). The

FCT and FEDER (European Fund for Regional Development)–COM-PETE/QREN/EU are acknowledged for financial support through the research unities PEst-C/QUI/UI686/2011 and PEst-OE/AGR/UI0690/2011, the research project PTDC/QUI- QUI/111060/2009 and the postdoctoral grant to R.C.C. (SFRH/BPD/68344/2010).

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P024

In Silico Design of Neuroplasticity Modulators

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Neuroplasticity, defined as the changing of the structure, function, and organization of neurons, has emerged as an interesting target for the development of new and effective treatments for multiple neurodegenerative diseases.

In this study, we have applied our proprietary computational platform, Symmetry[®], to assess more than 30 molecular and cellular targets involved in neuroplasticity and start designing potential small-molecule modulators.

This chemoinformatics technology is applied on top of large amounts of factual data and is able to characterize multiple molecular mechanisms of action and other important pharmacological endpoints. The system enables the generation of focused libraries covering a wide range of chemical diversity patterns around specific conditions, mechanisms of action or selected chemical scaffolds.

The screening of generated virtual compounds has demonstrated a strong correlation between predicted and real mechanisms of action, along with a convenient ADMET profile.

The corresponding synthesis and experimental validation has led to a series of small-molecule BDNF modulators which have been selected for further pharmacological evaluation.

P025

1-Aryl-3-[4-(thieno[3,2-d]pyrimidin-4-yloxy)phenyl]ureas as VEGFR2 Tyrosine Kinase Inhibitors: Synthesis, Docking Studies, Enzymatic and Cellular Assays

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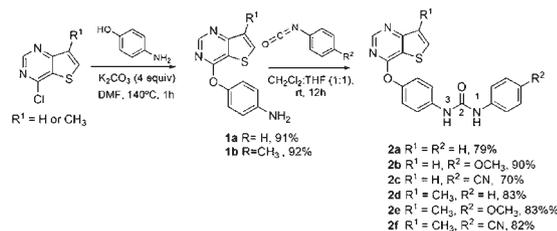
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A number of thienopyrimidines derivatives have shown potent vascular endothelial growth factor receptor 2 (VEGFR2) inhibition activity.^[1] Here, we present the synthesis of new 1-aryl-3-[4-(thieno[3,2-d]pyrimidin-4-yloxy)phenyl]ureas by promoting the regioselective attack of the hydroxy group of the 4-aminophenol in the chlorine nucleophilic displacement on two 4-chlorinated thieno[3,2-d]pyrimidines, obtaining compounds **1a** and **1b** which were reacted with arylisocyanates to give the corresponding 1,3-diarylureas **2a-f** (see scheme).

These compounds were evaluated for inhibition of VEGFR2 tyrosine kinase activity using enzymatic assays, and **2a-c** showed good inhibition ability with IC₅₀ values in the range of hundreds of nanomolar. The rationale for the inhibition activity is also discussed using docking. To examine the activity of **2a-c** in endothelial cells, human umbilical vein endothelial cells (HUVECs) were cultured in the presence or absence of each compound in different concentrations. A decrease in the proliferation of HUVECs was observed by the incorporation of BrdU quantified by ELISA assay. Given the established role of VEGFR2 in proliferation and migration of endothelial cells, these molecules are promising antiangiogenic agents that can be used for therapeutic purposes in pathological conditions where angiogenesis is exacerbated, such as cancer.



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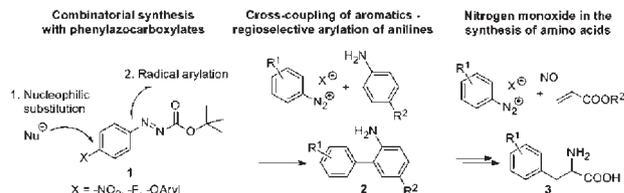
P026

Green Chemistry in Pharmaceutical Research—Inspirations from Radical Reactions

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Chemical transformations in medicinal chemistry and pharmaceutical manufacturing have to meet ever more complex demands such as sustainability and selectivity in order to be applied to the multifaceted challenges of today.^[1] To address these issues, radical chemistry has been a largely neglected discipline. Herein, we would like to present three recent examples showing the suitability of metal-free radical reactions for pharmaceutical purposes. Phenylazocarboxylates **1**, which are valuable building blocks for combinatorial synthesis, can be modified by nucleophilic substitution and radical reactions under mild conditions.^[2,3]



The synthesis of versatile 2-aminobiphenyls **2** has been achieved via a highly regioselective Gomberg-Bachmann arylation.^[4] Through a new type of the Meerwein arylation, nitrogen monoxide can be used for the preparation of aromatic amino acids **3**.^[5] This process is also a potential tool for the recycling of NO occurring as waste gas on multi-ton-scale every day.

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P027

Benzopyrone as a Privileged Scaffold for Drug Discovery: Synthesis and Structure–Affinity Relationships for Adenosine Receptors

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Despite the advances in medical and pharmaceutical sciences, there are still many diseases which are incurable maladies. Therefore, there is still a great need for more active and selective drugs with fewer undesired or toxic side-effects.

Adenosine is a purine nucleoside that modulates a variety of physiological and pathophysiological processes, mainly through the interaction with four subtypes of cell-surface G-protein coupled adenosine receptors (ARs), named A_1 , A_{2A} , A_{2B} and A_3 receptors. In fact, a multiplicity of physiological actions can be ascribed to adenosine including effects on heart rate and atrial contractility, vascular smooth muscle tone, release of neurotransmitters, lipolysis, renal, platelet and white blood cell functions. The recent findings of adenosine involvement in cancer and various CNS dysfunctions has led to the importance of developing and designing available selective AR ligands. A considerable number of selective agonists and antagonists of adenosine receptors have been discovered, and some have been clinically evaluated, although none has yet received regulatory manly due to their side effects, low absorption, short half-life and toxicity of the compounds. Therefore the aim of this project is the design and synthesis of a library of novel adenosine ligands that incorporate benzopyrone substructure. In order to identify the hypothetical binding modes at both the crystallographic structure of human AR a molecular modelling investigation of the newly synthesized analogues was also performed. The mentioned analysis was also extended to docking simulations and per residue electrostatic and hydrophobic contributions. The overall data will be presented in this communication.

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P028

Biotinylated Tryptophan Catabolites for Target Fishing

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Molecular recognition is at the heart of all biological interactions although its principles are not fully understood. Currently, the best approach to study and understand biorecognition is to determine the three-dimensional structure of the biomolecular complex experimentally via X-ray crystallographic methods or NMR. When the application of these methods is difficult, alternative systems are used, of which the most versatile is the avidin-biotin complex.^[1] An exciting application of this system is its use for the identification of the molecular target of small molecules (target fishing).^[2–4] A small molecule-biotin conjugate displaying the same properties as the original nonconjugated molecule could provide an opportunity to identify the target and study the interactions between the ligand and its cellular targets in great details.

In this context, we have been involved for some time in the biotinylation of tryptophan catabolites with the aim to dissect the molecular mechanism underlying their immunoregulatory effects. Indeed, preliminary data^[5] indicate that a single administration of L-kynurenine (L-Kyn) to female nonobese diabetic (NOD) mice with overt type 1 diabetes (T1D) counteracts the disease. These exciting results lay the foundation for a potentially efficient therapy for a real cure of T1D. L-Kyn is formed by metabolic degradation of L-tryptophan along the kynurenine pathway in which indoleamine 2,3-dioxygenase (IDO) catalyzes the initial rate-limiting step.

L-Kyn is then transformed by downstream enzymes into 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), and quinolinic acid (QUIN), collectively known as Kynurenines (Kyns). Among Kyns, 3-HAA was initially selected for its simpler structure with respect to L-Kyn, together with its known immunoregulatory role. To minimize steric hindrance and maximize binding, despite the presence of the bulky biotin moiety, a spacer arm was inserted between the ligand and the biotin molecule. The first realized biotinylated 3-HAA showed the same activity as the original nonconjugated molecule, and preliminary biorecognition experiments suggest that it specifically binds, both at the membrane and intracellular levels, different T cell subsets.

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P030

Novel Antithrombotic Compounds with Dual Activity

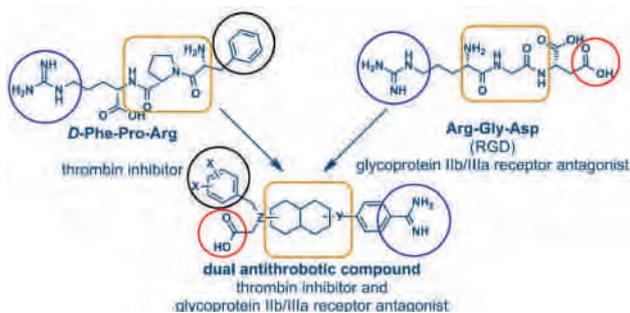
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The main research activity in the field of discovery of new antithrombotic agents is devoted to new anticoagulants and antiplatelet drugs. The development of effective and patient friendly antithrombotic agents remains a permanent challenge to medicinal chemists.

The rational design of compounds with designed multiple mode of action towards multiple targets is becoming a widely used approach in drug design. In the field of antithrombotic drugs several multiple ligands were published, however, they were mainly working on the similar targets (eg. fXa and thrombin). We developed for the first time compounds possessing thrombin inhibitory activity and fibrinogen receptor antagonism as novel antithrombotic drugs, combining enzyme and receptor as molecular targets.

Benzamidinium moiety was used for the P1 part of the molecule; various heterocycles were used as central scaffold, aromatic P3 moiety was optimized using various fluorine substituents on aromatic ring, and P4 carboxyl group moiety was optimized using optimal substitution on heterocyclic ring and the length of the alkyl chain. In the case of 1,4-benzodioxins both 6- and 7- regioisomers and enantiomers were prepared giving the insight into stereochemical requirements for balanced anticoagulants and antiplatelet activity. Animal studies were performed to demonstrate *in vivo* activity. Thus we are presenting compounds having nanomolar thrombin inhibitory activity as well nanomolar fibrinogen receptor antagonistic activity as novel antithrombotic compounds and potential drug candidates.



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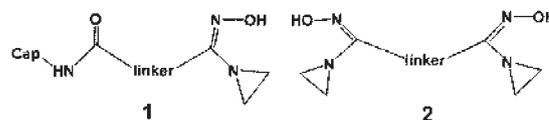
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P031

Synthesis and Structure–Activity Relationships of Aziridin-1-yl Oximes as Antitumor Agents

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Aziridin-1-yl oxime-containing molecules **1** caught our attention as potential antitumor agents. The properties of similar class of compounds **2** (bis-aziridine oximes) have recently been exploited and showed a high cytotoxic activity against cancer cell lines, however low *in vitro* LD₅₀ values.^[1,2]

On the basis of previous results, series of aziridin-1-yl oximes **1** were synthesized to evaluate their cytotoxic activity. The synthetic routes toward desired compounds were established and the modification of the cap and linker was realized.

New compounds were tested on several cancer cell lines and for intercalation with DNA strands. The results obtained allowed us to make preliminary conclusions about structure–activity relationships as well as provide hypothesis for further structure optimization of aziridin-1-yl oximes **1**.

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P032

P-TEFb Inhibitors as New Potential Anti-HIV Agents: Hit-to-Lead Optimization

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The emergence of multidrug-resistant viral strains along with the inability of the current drug regimen to completely eradicate the virus in the HIV-infected individuals demands new drugs capable of interfering with alternative targets or steps of the viral replicative cycle. An appealing strategy could be the interference with host factors involved in the Tat-mediated transcription. Among them, positive transcriptional elongation factor b (P-TEFb), composed by the cyclin-dependent kinase CDK9 in association with the regulatory subunit cyclin-T1, plays a pivotal role in sustaining high levels of HIV transcription. Indeed, it is hijacked by the viral protein Tat to the nascent stem loop TAR RNA, thus resulting in the resumption of productive elongation, after the phosphorylation of both the RNAPII CTD and negative transcriptional elongation factors.

Several experiments validated CDK9 as a druggable component of the P-TEFb complex.^[1–3] However, no inhibitor was rationally designed to fit this target selectively. Indeed, all of the known anti-HIV CDK9 inhibitors were retrospectively identified by screening anticancer agents toward a panel of CDKs. In order to identify innovative CDK9 inhibitors, we have recently performed structure-based drug design (SBDD) using the crystallographic structure of P-TEFb in complex with flavopiridol, the most potent CDK9 inhibitor.^[4] The multistep virtual screening followed by the antikinase activity determination led to the identification of some real hits able to inhibit CDK9 at nontoxic concentrations.^[5]

Starting with one of the best molecules, characterized by a quinazolinone fragment, a series of analogues has been realized by applying two first cycles of optimization. In this presentation, the design, synthesis, anti-CDK9 and cytotoxic evaluation along with the ability to inhibit the Tat-mediated transactivation and HIV replication for the best molecules will be reported.

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P033

Novel Pyridazinone Analogues with Potential Activity on the Cardiovascular System

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Pyridazinone derivatives have received increasing interest in medicinal chemistry due to their important pharmacological properties, particularly on the cardiovascular system as antiplatelet and vasorelaxants agents.^[1] In a previous work, we developed different series of 2- and 2,6-substituted pyridazin-3(2H)-ones with vasorelaxant and platelet antiaggregatory activities in the micromolar range. A preliminary study of structure–activity relationship suggests that both effects would be enhanced by an increase in the lipophilicity on the pyridazinone ring.^[2] For this reason and also in order to analyze the importance of substitution at C6, we have designed new series of compounds showing the following structural features: 1) an extra methyl group at C5; 2) the side chain at C5 instead at C6; 3) an additional ring linking the C5 and C6 positions.

The synthetic strategy followed to build the pyridazinone core was based on oxidation of alkyl furans with singlet oxygen to give a functionalized butenolide suitable to react with hydrazine or substituted hydrazines.^[3] Finally, standard procedures allow us to obtain the pyridazinone derivatives with the desired substituent in the alkyl chain. The synthesized compounds were tested as antiplatelet and vasorelaxant agents and their pharmacological data will be discussed.



Figure 1. General structure of pyridazinones synthesized.

Acknowledgements: We acknowledge the Universidade de Vigo (Spain) for financial support and for a predoctoral contract (T.C.).

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P034

***Ganoderma lucidum* Methanolic Extract: Chemical Characterization in Phenolic Compounds and Study of Growth Inhibitory Activity in Human Tumour Cell Lines**

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Ganoderma lucidum is one of the most extensively studied mushroom species due to its medicinal properties. It has been used as functional food and as chemopreventer in some countries for thousands of years, and became a popular dietary supplement ingredient in Western countries.^[1] Some of its pharmacological properties have been related to antitumour properties, attributed to a wide variety of bioactive components such as polysaccharides, triterpenes, sterols, lectins and some proteins.^[2,3] Nevertheless, the bioactive properties of its phenolic compounds have not been studied. The aim of this work was to study the potential antitumor activity of the methanolic extract of this mushroom. This extract of *Ganoderma lucidum*, collected in Northeast Portugal, was characterized in phenolic compounds by high performance liquid chromatography coupled to photodiode array detection and mass spectrometry (HPLC-DAD-MS). The extract was further submitted to evaluation of growth inhibitory activity in four human tumour cell lines (MCF-7, NCI-H460, HCT15 and AGS), by the sulforhodamine B assay.

The extract presented a moderate growth inhibitory activity in all the cell lines tested ($GI_{50}=93.3 \pm 18.1-112.6 \pm 11.7 \mu\text{g/mL}$). The following compounds were identified in the extract: *p*-hydroxybenzoic acid ($0.58 \pm 0.04 \text{ mg/100 g dw}$), *p*-coumaric acid ($0.38 \pm 0.03 \text{ mg/100 g dw}$) and cinnamic acid ($0.28 \pm 0.03 \text{ mg/100 g dw}$). Future work will elucidate the mechanism of action of the studied extract leading to the observed cell growth inhibition.

Acknowledgements: Funding from the FCT and FEDER COMPETE/QREN/EU through project PTDC/AGR-ALI/110062/2009 and through the research centres (PEst-C/QUI/UI0686/2011 and PEst-OE/AGR/UI0690/2011) is acknowledged. S. A. Heleno also thanks the FCT for a PhD grant (SFRH/BD/70304/2010).

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P035

Indication of Brain Penetrable Compounds in Plant Extracts by PAMPA-BBB–LC-MS Assay

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Plant extracts with proven neurological bioactivity are attractive and potential targets for central nervous system (CNS) drug discovery. In the vast majority of cases, however, the molecular mechanism of action and the constituents responsible for activity remain unclear or uncertain due to the complexity of natural products. To overcome this issue, predicting and evaluating the blood–brain barrier (BBB) permeability of natural products is of key importance. Parallel artificial membrane permeability assay (PAMPA) is a robust, 96-well plate assay-based in vitro method for assessing the rate of transcellular passive permeability of drug candidates through the BBB. The goal of our study was to validate the applicability of the PAMPA-BBB assay coupled with LC-MS for identifying brain penetrable compounds in really complex mixtures. Our validation set contained 43 natural product drugs and natural product-like drugs with experimental blood–brain partition coefficients ($\log_{BB}=\log(C_{\text{brain}}/C_{\text{blood}})$) ranging evenly from -2.0 to 1.0 in value. In order to measure the effective permeability (P_e) and membrane retention (MR%) of each test compound, rapid LC-MS methods were developed. Finally, we demonstrate the applicability and advantages of PAMPA-BBB assay with the extract of *Corydalis cava* and *Tanacetum parthenium*, containing several CNS active benzyloquinoline alkaloids and sesquiterpene lactones, respectively.

P036

Fluorescent Probes for Acetylcholine Detection
In Vivo

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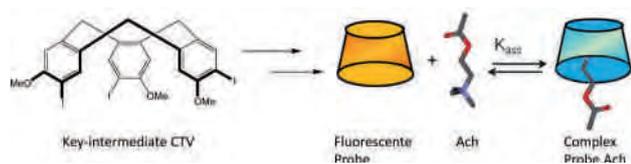
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The development of fluorescent probes for the in vivo detection of neuronal species presents a growing interest, as they can be efficient tools to investigate the mechanisms involved in neurodegenerative diseases.

In this context, in our group, we develop fluorescent probes having a cyclotriveratrylene (CTV) skeleton for the detection of acetylcholine (Ach) and its precursor and metabolite choline (Ch).^[1,2]



CTV are bowl-shaped structures known to complex quaternary ammoniums like acetylcholine.^[3,4] CTV can be fluorescent via photo-induced charge transfer (PCT) if conjugated with withdrawing and donating groups are introduced onto the aromatic skeleton. Complexation of Ach by the CTV leads to a modification of the CTV fluorescence properties. Up to now, none of the fluorescent CTV probes fulfill all the criteria required for an in vivo application (solubility in biological medium, high excitation wavelength, and selectivity for acetylcholine especially versus choline). Introduction of more suitable withdrawing groups (like phosphonic acid or acid) onto the aromatic skeleton improve the probe properties, such as solubility in water. Increasing the conjugation between the donating and the withdrawing groups using organometallic coupling reactions, we obtain a deeper hydrophobic cavity, with good fluorescence properties (excitation wavelength, quantum yield) and interesting affinity for acetylcholine. In order to introduce various functionalities we have elaborated a new convergent strategy from a key-intermediate CTV, bearing iodine groups.^[5]

In this communication, we will present first, the versatile syntheses of the key-intermediate CTV and the new fluorescent CTVs obtained. Then, we will concentrate on the spectroscopic properties and the detection results, like the affinity and selectivity towards Ach. Finally, we will present the results obtained using our probes in living neurons.

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P037

pH-Gradient PAMPA-Based In Vitro Model Assay
for Drug-Induced Phospholipidosis in Early Stage
of Drug Discovery

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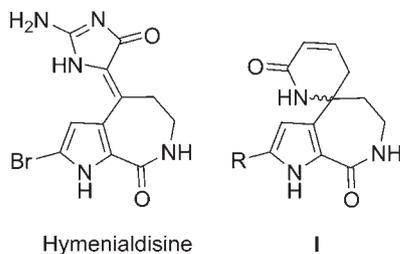
Drug-induced phospholipidosis (D-PLD) is a lipid storage disorder characterized by the excessive accumulation of phospholipids within lysosomes and the inducing drug into the typical drug-phospholipid complex in affected tissue. Several mechanisms have been postulated for D-PLD: 1) accumulation of a CADs and subsequent formation of a drug-phospholipid complex resistant to degradation by phospholipase within lysosomes, 2) direct inhibition of phospholipase in the cytosol and 3) inhibition of intracellular pathway of phospholipid metabolism. Fundamentally, the most critical step of the lysosomal dependent D-PLD formation is the one-way transport of CADs, which occurs by the following: non- or partially ionized amphiphilic amines (CADs) present in the cytosol (~pH 7.4) penetrate into the lysosomes (~pH 4.0–5.0), become protonized and in accordance with Brodie's pH partition hypothesis trapped in the acidic milieu. In this report, we describe a new approach for prediction of D-PLD with in vitro noncell base permeability system. We measure a drug–lipid complex formation and also a drug transport to lysosomes via two characteristic physicochemical parameters of the novel pH-gradient PAMPA system, namely membrane retention (MR) and effective permeability (Pe). Next to Millipore's two 96-well plate sandwich-based PAMPA system, the instrument required is a LC-UV system with a plate sampler to analyze evolving concentrations of compounds at two side of permeability system, which could ensure effectively high-throughput capacity for indication PLD potential of candidates in early stage of drug discovery.

P038

Novel Hymenialdisine Derivatives as Potential CDK Inhibitors

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Protein kinases comprise components of many signal transduction pathways related with vital biological processes.^[1] Their hyperactivation is common in various diseases such as cancer, inflammation, neurodegenerative and metabolic disorders. Thus, they represent promising molecular targets for the treatment of these diseases. During the last years, great efforts have been directed to discover novel small molecules with specific protein kinase inhibitory activities.^[2] Among other protein kinases which are involved in the cell cycle regulation and transcription are cyclin-dependent kinases (CDKs).^[3] Their implication in pathological disorders such as cancer^[4] led to the discovery of many small heterocycles as either broad-range or selective ATP-competitive CDK inhibitors.^[5]

Marine natural product (HMD) has been shown potent inhibitory activity against various kinases such as CDKs, GSK-3 β and CK1.^[6] Structurally, it consists of a pyrroloazepine skeleton connected to a glycoamidine ring. Both, these two structural components ensure the effective binding of HMD on the ATP-binding site of targeted kinases.^[6] Although HMD exhibits CDK inhibitory activity in the nanomolar range, the discovery of novel analogues with better selectivity profile remains an open challenge.

In continuation of our efforts in the field of CDKs,^[7] we present in this communication the design and synthesis of novel spiro-HMD derivatives (**I**) as potential CDK inhibitors. The new compounds incorporate a functionalized pyrroloazepine core and a spiro six-membered lactam ring system. Our synthetic approach provides access to the desired target compounds in *enantiomerically* pure forms. The investigations towards the synthesis of the key intermediates and the target compounds will be described.

Acknowledgements: This study was financial supported by a grant "K. Karatheodori" (C.910) through the Research Committee of the University of Patras, Greece.

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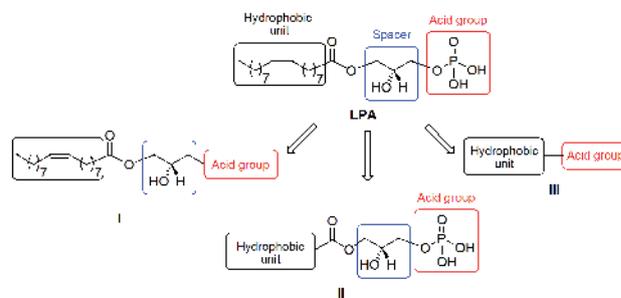
P039

Development of New Ligands for the Validation of the Lysophosphatidic Acid Receptor LPA₁

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Validation of therapeutic targets is nowadays a prior objective, as the need of new targets to face unmet clinical needs is constantly increasing.^[1] In this aspect, G protein-coupled receptors (GPCRs), which constitute around 50% of the druggable genome, stand out as a suitable family for the development of new drugs.^[2] Among them, former orphan Edg2 receptor has been recently characterized as the lysophosphatidic acid (LPA) receptor of type 1 (LPA₁ R). Given the key role of LPA in the central nervous system,^[3] the need for selective and high-affinity ligands of LPA₁ R is critical for the validation of this receptor.



Herein, we present the design, synthesis and biological evaluation of three series (**I–III**) of new compounds based on the structure of the endogenous ligand LPA with the objective of identifying new LPA₁ R ligands. These results should provide the basis for further biological studies to enlighten the role of LPA₁ R in human physiology.

Acknowledgements: This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and the Comunidad Autónoma de Madrid (SAL-2010/BMD2353). The authors thank MINECO for a predoctoral grant to I.G.-G., and MINECO and European Social Fund for a Ramón y Cajal grant to S.O.-G.

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P040

Validation of FtsZ Protein as a New Potential Therapeutic Target for the Discovery and Development of New Antibacterial Agents

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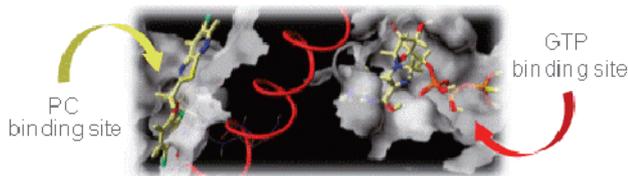
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Emergence and spread of antibiotic-resistant strains of pathogenic bacteria have boosted an urgent need for new antibacterial agents with novel modes of action. In this sense, FtsZ, "a widely conserved tubulin-like GTPase", has recently been proposed as an attractive target for antibacterial drug discovery due to its essential role in bacterial cell division.^[1]

Recently, several small molecules that specifically target FtsZ and inhibit its function in bacterial division have been identified.^[2] Among them, the most promising FtsZ inhibitor discovered so far is PC190723.^[3,4] This compound binds an alternative site different from the classical GTP binding site^[5] and has shown potent activity both in vitro and in vivo against *Staphylococcus aureus* but it is inactive against a range of Gram-positive and Gram-negative pathogenic bacteria. Hence, the development of new inhibitors of FtsZ able to act as broad-spectrum antibacterials needs still to be addressed and will be the focus of the present work.



Therefore, the main goal of this project is the discovery of FtsZ inhibitors targeting both binding sites, using two different strategies: the design of GTP-mimetics and virtual screening. In addition, synthesis of fluorescent derivatives of PC190723 is being carried out to obtain a valuable tool to set up a fluorescent assay which would allow for the assessment of the affinity of new synthesized compounds for this recently identified binding site.

Acknowledgments: This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and the Comunidad Autónoma de Madrid (SAL-2010/BMD2353). The authors thank MINECO for a predoctoral grant to M.E.A.

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P041

LYP Inhibits T cell Activation when Dissociated from CSK—Implications for a Novel Therapeutic Strategy in Autoimmunity

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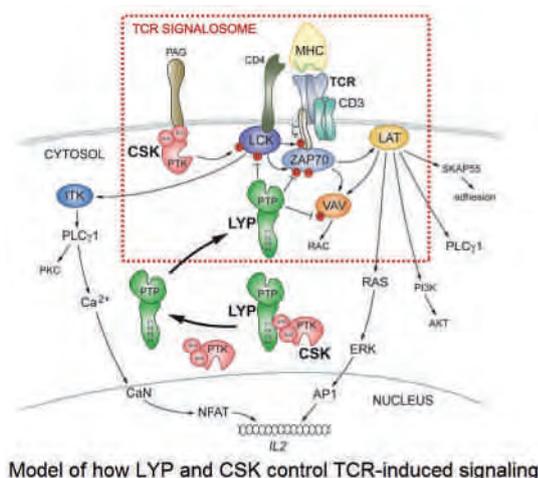
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A dynamic balance between tyrosine phosphorylation and dephosphorylation of signaling molecules is crucial for maintaining the homeostasis of the immune system. In T cells, T cell antigen receptor (TCR) stimulation leads to mobilization of the Src family kinase LCK, which initiates a cascade of phosphorylation events, ultimately resulting in the expression and release of stimulatory cytokines. TCR-induced responses are transient, and different mechanisms are involved in signal termination, including phosphorylation of LCK on its negative regulatory residue Tyr505 by the C-terminal Src kinase CSK, and dephosphorylation of its positive regulatory residue Tyr394 by the lymphoid tyrosine phosphatase LYP.

A single-nucleotide polymorphism (SNP) in the LYP gene PTPN22 (C1858T) correlates with the incidence of various autoimmune disorders. In fact, in populations of European descent, PTPN22 currently ranks third and second in terms of single-gene contribution to the etiology of type 1 diabetes and rheumatoid arthritis, respectively. The SNP results in alteration of Arg620 in the 'normal' allele (LYP*R620) to tryptophan in the disease-associated allele (LYP*W620). Residue 620 is located in the first of four proline-rich motifs that are found on the C-terminal part of LYP. Interestingly, Arg620 is crucial for the interaction between LYP and the CSK-SH3 domain, rendering LYP*W620 incapable of binding CSK. Experiments with primary T cells have indicated that LYP*W620 is a gain-of-function mutant that has approximately 50% higher catalytic activity and acts as a more potent inhibitor of TCR signaling. Since the risk allele LYP*W620 cannot bind CSK and is a stronger inhibitor of TCR

signaling, we hypothesized that the interaction between CSK and the major allele LYP*R620 could interfere with the catalytic duties of the latter.

To test our hypothesis, we studied the spatiotemporal dynamics of the LYP/CSK complex in human T cells. We demonstrate that dissociation of this complex is necessary for recruitment of LYP to the plasma membrane, where it down-modulates TCR signaling. Development of a potent and selective chemical probe of LYP confirmed that LYP inhibits T cell activation when removed from CSK. Our findings may explain why the risk allele LYP*W620 is a more potent inhibitor of TCR signaling and suggest a positive regulatory role for the pool of CSK molecules that interact with LYP. Our compound also represents a starting point for the development of a LYP-based treatment for autoimmune diseases and provides a new tool for further studies aimed at elucidating how LYP contributes to the development of autoimmunity.



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P042

Locally Acting ROCK Inhibitors for the Treatment of Glaucoma: From Design to Clinical Candidate

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ROCK is a downstream effector of the small GTPase Rho. Inhibition of ROCK induces several effects of pharmacological interest, such as relaxation of vascular smooth muscle fibers or alteration of the

intracellular junctions in the trabecular meshwork of the eye. The outstanding therapeutic potential of ROCK inhibitors is currently largely unexploited, because systemic inhibition of ROCK leads to strong biological effects that are considered side effects for the treatment of most diseases. ROCK inhibitors are however of interest for the treatment of conditions such as glaucoma. Topical administrations for this blinding disease are highly preferred. Therefore, specific medicinal chemistry approaches towards localized drug action strategies are of great interest to obtain safe and effective drugs. We here report the design and evaluation of locally acting ROCK inhibitors as drug candidates for the treatment of glaucoma.

Modification of Y-27632 resulted in a new series of potent ROCK inhibitors. Occupancy of a vacant space under the P-loop (glycine-rich loop) yielded compounds with significantly improved on-target potency. A nearby solvent-exposed cleft provided an attractive opportunity for the introduction of functional groups of interest for the development of locally acting inhibitors. In particular, we observed that introduction of ester-containing chains, which are potential substrates for blood esterases, was tolerated, yielding compounds with potent on-target and functional activity. Such compounds can be rapidly hydrolyzed once they leave the target organ and enter the blood flow, resulting in metabolites with negligible functional activity. Further optimization of this compound series resulted in the discovery of AMA0076, a locally acting ROCK inhibitor displaying strong in vivo activity and reduced systemic exposure. Further development of AMA0076 is currently on-going.

P043

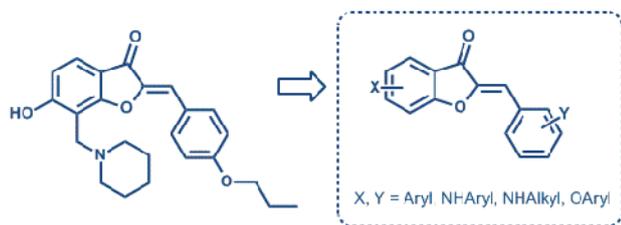
Development of Novel Aurone Derivatives as Potential Antimalarial Agents

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Drug resistance to currently established antimalarial drugs such as chloroquine (CQ) is increasing global mortality due to malaria.^[1] This disease is responsible for an estimated 225 million clinical cases and one million deaths annually,^[2] and therefore, novel and innovative inhibitors active against *Plasmodium falciparum*, which produces the most aggressive form of malaria, are urgently required in order to develop new treatments able to fight malaria.^[3]

Aurones are secondary metabolites belonging to the flavonoids family, and their antimalarial activity was already recognized.^[4] More recently, it was shown that the mechanism of action of this family is most likely a CQ-like action, i.e., by inhibiting the hemozoin (malaria pigment) formation inside the acidic digestive vacuole of the parasite.^[5] Degradation of hemoglobin by malaria parasite proteases causes the release of ferriprotoporphyrin IX (FPIX), which is detoxified by crystallization to hemozoin in the digestive vacuole. CQ and related antimalarial drugs bind to FPIX via π - π stacking of the aromatic moiety with the porphyrin ring, thus inhibiting detoxification.^[6]



In an attempt to obtain new potent antimalarial agents and explore the chemical space around this scaffold, a library of novel aurone derivatives was synthesized by introducing an additional aromatic moiety by using Suzuki–Miyaura and Buchwald–Hartwig cross-coupling reactions. The synthetic procedures and some preliminary results will be presented.

Acknowledgments: This work was financially supported by the Fundação para a Ciência e Tecnologia (FCT, Portugal) through the projects PTDC/SAU-FCF/098734/2008 and PEst-OE/SAU/UI4013/2011. FCT is also acknowledged for the Ph.D. grant SFRH/BD/61611/2009.

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P044

Unraveling the Molecular Basis for DAF-12 Activation: Diastereoselective Synthesis and SAR Studies of Dafachronic Acid Derivatives

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Upon unfavorable conditions and difficult environmental plights, several organisms from worms to mammals respond by arresting their development and initiating programs of reversible states of dormancy.^[1] Many strategies of diapause have evolved even within the same species, allowing them to survive until conditions improve and they can return to normal reproductive life.

Studies on the hibernation-like stage of the nematode *Caenorhabditis elegans* (dauer diapause, L3) have provided crucial insights into the diversity and complexity of these alternate life strategies.^[2] In particular, it was reported that steroid hormones called dafachronic acids (DAs) promote dauer recovery through the activation of the nuclear hormone receptor DAF-12.^[3] Remarkably, recent evidences support the hypothesis that the same pathway is shared by parasitic nematodes and that DA-like compounds can break off the infectious cycle before parasites are in the host environment needed for them to complete the life cycle.^[4] These important findings reveal a new therapeutic direction to treat a wide range of nematode infections, which affect more than 1 billion people worldwide, as well as pathogenic infestations of livestock and plants.

On the basis of these considerations and with the aim to better define the biological relevance, endocrine circuitry and molecular mechanism governing the action of DAF-12, we report the diastereoselective synthesis,^[5] biological appraisals, and structure–activity relationships of a series of DA derivatives as novel DAF-12 ligands. The results revised in the light of computational analysis have provided further mechanistic insights into the molecular features of the receptor activation and may be useful in designing and identifying species-selective DA-based modulators.

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P045

Finding Right Targets for Drug-Like Compounds by Computer-Aided Prediction of Biological Activity

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Computer program PASS predicts over 4300 kinds of biological activity with mean accuracy about 95% based on the analysis of the training set with information about ~250000 pharmaceutical agents and biologically active compounds. Since PASS predicts simultaneously interaction of chemical compounds with a large number of biological targets, based on the prediction results with computer program PharmaExpert it is possible to select the compounds with pleiotropic action. In such way we found new antihypertensive and antiinflammatory pharmaceutical agents with dual mechanisms of action,^[1,2] and also we discovered nootropic effects in antihypertensive drugs, which are not caused by their antihypertensive action.^[3]

We developed a freely available web service (<http://pharmaexpert.ru/passonline>), which allows obtaining prediction of biological activity spectra via the internet. The web service is utilized by ~7500 users from ~60 countries. In dozens of cases, the prediction results for drug-like compounds belonging to different chemical series and having various kinds of biological activity were confirmed by further experiments. For instance, the following biological activities were predicted and shown in biological assays: antiarrhythmic activity for 2-diethylamino-2',6-dimethylphenylacetamide derivatives;^[4] anti-inflammatory and antibacterial actions for glycoside quercetin;^[5] cytotoxic and clastogenic actions for 3,6-di-substituted acridines;^[6] trichomonocidal, giardicidal and amebicidal actions for *N*-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles;^[7] anti-diabetic activity of flavonoids;^[8] etc.

Therefore, based on PASS predictions, it is possible to identify the most probable targets/effects for the compounds under study.

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P046

Antimicrobial Characteristics of Nanochitosan-Treated Wool Fabric Dyed with Weld Natural Dye

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Colorful antimicrobial fabrics are used in the manufacturing of garments and gowns in hospitals as they generate a positive vibe than other plain fabrics. Chitosan, a common natural biopolymer, is being used on fabrics as a finishing treatment to achieve antimicrobial characteristics. In this study, the particle size of this polymer is decreased into nanoparticles using two separate processes. Final size of two nanochitosans was measured. Then each of these two nanochitosans was separately applied on wool fabric to investigate their effect on the modification of protein fibers. Antimicrobial properties were studied for them. In next step, the effect of concentration of nanochitosan as an antimicrobial material was detected. Then, wool fabric was dyed using a natural folklore dye called "Weld". Antimicrobial properties of dyed nanochitosan-treated fabric were investigated. As the last step, wash fastness of the samples was measured.

Two methods were used to reduce the size of chitosan. The main method was "coacervation" for chitosan. In method 2 before this, H₂O₂ was used for polymer degradation. DLS results showed that sample 2 has smaller particle size in the nano range. Different concentrations of nanochitosan sample 1 were applied to the fabric and antimicrobial properties for all were measured. Results shows that nanochitosan sample 1 (without H₂O₂) shows better antimicrobial properties and the higher concentration gives the most reduction in bacteria number.

"Weld" had been used in cosmetics before. Treated fabrics were dyed with weld natural dye to investigate the antimicrobial properties of them. Result shows that weld can act as an antimicrobial agent against Gram-positive bacteria like *S. aureus* but combination of treating the fabric with nanochitosan and dyeing it with weld decreases the antimicrobial properties of samples.

Wash fastness of samples was measured to see the effect of treatment on it. Treatment with nanochitosan did not have any negative effect on fabric fastness.

Table 1. Microbial reduction (R) values of dyed nanochitosan-treated fabrics against *S. aureus* bacteria.

R [%]	(B-A)/B	B-A	A=T1	B=T0	Sample (nanochitosan concn)
53.00	0.53	5300	4700	10,000	0
38.00	0.38	3800	6200	10,000	0.5
10.00	0.10	1000	9000	10,000	1
5.00	0.05	500	9500	10,000	1.5

P047

Design and Synthesis of New Inhibitors of the Enzyme Isoprenylcysteine Carboxyl Methyltransferase (ICMT)

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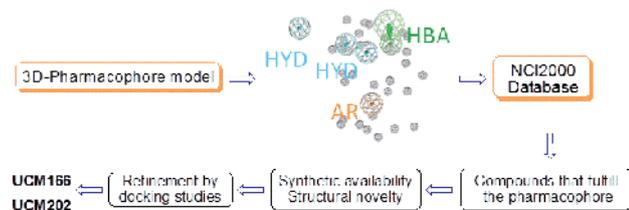
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Ras is a central component in many signal transduction pathways. Activating mutations in Ras have been found in almost 30% of all cancers, including 50% of colon cancers and up to 90% of pancreatic cancers.^[1] It has been demonstrated that in the absence of any of its post-translational modifications Ras loses its ability to induce tumor transformation. Therefore, the blockade of the enzymes involved in these modifications represents an attractive strategy to inhibit Ras activity. Among them, isoprenylcysteine carboxyl methyltransferase (ICMT)^[2] is receiving an increasing attention. To date, very few inhibitors structurally distinct have been disclosed, and only one molecule (cysmethynil) has been characterized as an ICMT inhibitor not only in vitro but also in cellular systems, where it blocks the anchorage independent growth in a human colon cancer cell line.^[3] These findings provide a compelling rationale for the development of ICMT inhibitors as another approach to anticancer drug development.



Towards this objective, we have addressed the design of new compounds with the elaboration of a 3D-pharmacophore model, which has been further refined based on the recently described crystal structure of a prokaryotic ICMT ortholog.^[4] From our initial series, we have already succeeded in identifying some hits with interesting ICMT inhibitory activities (UCM166 and UCM202, which inhibit a 84% and 93% of the control ICMT activity at 50 μ M, respectively). These results, which are guiding the hit to lead process in order to improve not only their potency at ICMT but also their ADME properties, will be presented.

Acknowledgements: This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and Comunidad Autónoma de Madrid (SAL-2010/BMD2353). The authors thank MINECO for a predoctoral FPI fellowship to M.B., and MINECO and the European Social Fund for a Ramón y Cajal grant to S.O.-G.

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P048

A Molecular Dynamics View on the Efflux Mechanism of P-Glycoprotein

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P-Glycoprotein (P-gp) is often involved in multidrug-resistance (MDR) to the pharmacological action of a wide number of anticancer agents.^[1] Herein, we present a series of molecular dynamics (MD) simulations of murine P-gp,^[2] elucidating the importance of the lipid membrane^[3] and linker sequence^[4] in the protein's structure and stability. The behavior of several molecules inside the drug binding pocket was studied and revealed a striking difference in the number, type and residues involved in substrate or modulator interactions. Motion patterns were also identified that could be correlated with conformational alterations due to substrate binding, corresponding to the initial step of the efflux mechanism. Only one 'entrance gate' to the drug binding pocket was found and, in the presence of a substrate, leads to alterations in the motion patterns of the transporter into an efflux-like movement.

Acknowledgements: This work was supported by the Fundação para a Ciência e Tecnologia (FCT) through project PTDC/QUI-QUI/099815/2008 and PEst-OE/SAU/UI4013/2011.

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P049

Thieno[3,2-*b*]pyridine Arylethers: Synthesis and Growth Inhibitory Activity on Human Tumor Cell Lines

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Thienopyridine skeleton has been reported as having interesting biological activity, namely antitumor^[1] and antiangiogenic^[2] activities. Herein, we describe the synthesis of thienopyridine arylethers **1a–f** in moderate to good yields by a copper-catalyzed C–O coupling, using *N,N*-dimethylglycine as a ligand, of the 7-bromothieno[3,2-*b*]pyridine, also prepared with substituted phenols (see scheme).



- 1a**, R¹=F, R²=R³=H 63%
1b, R¹=R³=H, R²=F 61%
1c, R¹=R²=H, R³=F 66%
1d, R¹=OMe, R²=R³=H 40%
1e, R¹=R³=H, R²=OMe 50%
1f, R¹=R²=H, R³=OMe 45%

The growth inhibitory activity of the di(hetero)arylethers **1a–f** was evaluated against four human tumor cell lines (MCF-7, NCI-H460, HepG2 and HeLa), using the sulforhodamine B assay. Furthermore, the hepatotoxicity of compounds was studied using a porcine liver primary cell culture (PLP1). The most promising compound was

shown to be the methoxy derivative (**1e**) presenting GI₅₀ values in the range of 1.5 to 6.5 μM. For this compound, more studies are needed to find its mechanism(s) of action.

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P050

Non-anionic Aldose Reductase Inhibitors. Design, Synthesis, Biological Evaluation and In Silico Studies

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Aldose reductase’s (ALR2) involvement on the onset and progression of diabetes secondary complications has attracted attention over the years.^[1] Furthermore, recent evidence point towards ALR2’s implication in inflammatory pathologies.^[2] As such, ALR2 comprises a compelling target for medicinal chemistry.

In the plethora of aldose reductase inhibitors (ARIs) synthesized so far, two categories are the most studied, namely that of cyclic imides and carboxylic acid derivatives. However, a number of cyclic imide derivatives emerged with acute side effects and carboxylic acids presented with poor membrane penetration.

In our previous work and in order to overcome the limitations of the two classic categories of ARIs, we have presented a successful bioisosteric replacement of a carboxylic acid moiety with that of a 2,6-difluorophenol.^[3,4] 2,6-Difluorophenol has a *pK_a* value of 7.12, therefore its derivatives could diffuse through membranes more adequately than their carboxylate counterparts. In the present work, we investigated the synthetic feasibility and ARI activity of aroylpyrroles bearing groups that are non-anionic in physiological pH such as the phenol, 2-fluorophenol, salicylaldehyde, nitroaldehyde, and 3,4-di-

fluorophenyl moiety. The 2-fluorophenol derivative exhibited the most promising combination of activity and physicochemical properties, thus a further optimization of this structure was exploited.

In contrast to the prevalent notion that anionic species inhibit ALR2, we found that a number of the prepared 2-fluorophenol derivatives are active inhibitors of ALR2 with IC_{50} values in the low micromolar range. The synthetic routes and structure–activity relationships of these novel hit compounds, along with their selectivity to the homologous enzyme aldehyde reductase (ALR1), are discussed in terms of structural properties and in silico studies. Moreover, in an effort to evaluate the ability of the novel derivatives to penetrate through membranes, key physicochemical properties are calculated as well as experimentally measured.

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P051

Synthesis and Biological Testing of 5-Pyridinyl-2-thioimidazole Derivatives to Gain Selective JNK3 Inhibitors over P38 α

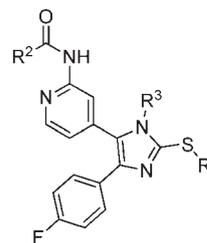
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Many inflammatory conditions are driven by both p38 α and JNK3 MAPK.^[1] To differentiate the contribution of each kinase, selective inhibitors are necessary. For p38 α this might be the case,^[2] but as of today only few selective JNK3 inhibitors with good absorption, distribution, metabolism, excretion (ADME) properties are available.

JNK-kinases are c-jun NH₂-terminal serine/threonine mitogen-activated kinases which are mainly activated by cytokines and environmental influences.^[3,4] JNK3 kinases are believed to play a central role in the pathology of neurologic diseases such as cerebrovascular accidents, Parkinson's and Alzheimer's disease.^[5] Therefore, it has become an attractive and valid drug target.

5-Pyridinyl-2-thioimidazole derivatives are known as p38 α inhibitors.^[6] Due to the sequential and steric similarity of p38 α and JNK3 kinase, we assumed to gain active and selective JNK3 inhibitors by introducing different substitution patterns for R¹, R², and R³.



At the edge of the hydrophobic region II in JNK3 kinase, there are Asn152 and Gln155, whereas Asp112 and Asn152 are shown in p38 α kinase.^[3] In order to create repulsion between the Asp112 and p38 α , we introduced anionic substituents at the aminopyridine scaffold. Furthermore, we tried to hit the Asp112^[3] by introducing carboxylic moieties at the imidazole nitrogen. In order to target the conserved but steric diverse Arg107 and Asn194,^[3] we synthesised carboxylic substituents linked by a sulfide at R¹ resulting in 50 nM inhibition of JNK3 with about 10-fold selectivity against p38 α .

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P052

New Indole Derivatives as Potential NMDA Receptor Antagonists

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The NMDA receptor is a complex ligand gated, voltage-dependent ion channel. Excessive activation of NMDA receptors induces the death of central neurons as a result of Ca²⁺ influx. So, aberrant NMDAR activity plays an important role in the neuronal loss associated with major degenerative disorders including Parkinson's and Alzheimer's disease.

The indole alkaloids hirsutine, and hirsuteine show inhibitory effects in NMDA receptors, increasing cell viability by suppressing NMDA-induced apoptosis.^[1] As a result, indole alkaloids or structurally related-alkaloids may serve as useful drugs for treatment and/or prevention of neurodegenerative diseases that involve excess stimulation of NMDA receptors. For this reason, we decided to extend our research in synthesis of tryptophan derived oxazolopiperidone lactams^[2] to synthesize libraries of indole derivatives to be evaluated as NMDA receptor antagonists.

We report here the synthesis of libraries of enantiopure L-tryptophan derived lactams **1** and **2** (Figure 1) to be evaluated as NMDA receptor antagonists. Due to the known potential differences in activity of enantiomeric series of biologically active compounds, the analogous series starting from the D-enantiomer of the original tryptophan precursor were also synthesized.

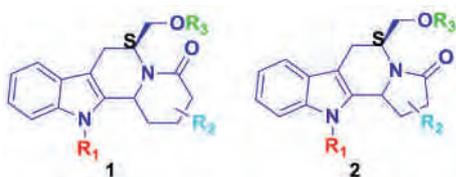


Figure 1. Libraries of Potential NMDA receptor antagonists synthesized.

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P053

Human Neutrophil Elastase: Virtual Screening Approach toward Lead Generation for COPD Drug Discovery

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Human neutrophil elastase (HNE) plays an important role in chronic obstructive pulmonary disease (COPD) inflammatory process where in an excess of HNE is produced hydrolyzing elastin, the structural protein which gives the lungs their elasticity. The available COPD therapeutic is limited to palliative drugs and no HNE inhibitor got FDA or EMA approval for the treatment of COPD. Besides active efforts

over the past 30 years to achieve efficient inhibitors of HNE, these were discontinued for various reasons. Hence, it becomes vital to design an effective HNE inhibitor.^[1] Herein we present a new approach to boost discovery of drug candidates for treatment of COPD relying on the use of structure-based screening of the molecular operating environment (MOE) drug-like database (Figure 1). A commercial library of 653214 drug-like compounds from different suppliers was docked into the HNE enzyme active site, and 28 compounds were selected for purchase and tested. Four new HNE inhibitors in the low micromolar range were identified, displaying selectivity towards HNE when compared with other neutrophil serine proteases. Moreover, the identified leads exhibited a noncytotoxic profile. One of these compounds was selected for further development and a library of compounds was synthesized and assayed against HNE.

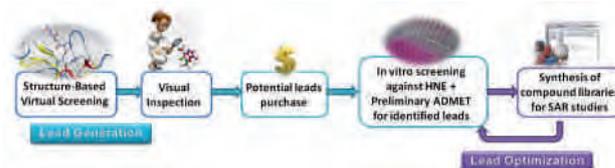


Figure 1. Workflow toward lead generation/optimization for COPD drug discovery.

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P054

Design, Synthesis and Evaluation of New Voltage-Gated Sodium Channel Modulators

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Voltage-gated sodium channels (Na_v channels) are integral membrane proteins composed of a circular arrangement of identical or homologous domains surrounding a water-filled pore. They play an essential role in the initiation and propagation of action potentials in neurons and other electrically excitable cells such as myocytes and endocrine cells.^[1,2] Na_v channels are composed of a single α-subunit, which forms a voltage-sensing pore, and one or more auxiliary β-subunits. To date, nine different α-subunits (Na_v 1.1–Na_v 1.9) and four different β-subunits have been identified. These subtypes have similar structures, but the expression of α-subunits is strongly cell- and tissue-specific, therefore each of these subtypes is believed

to have unique properties.^[3] The abnormally increased activity of sodium channels leads to over-excited state of specific groups of cells, which can cause different neurodegenerative diseases, chronic pain, epilepsy, arrhythmias, and spasticity.^[4] Although there are many drugs acting at Na_v channels, a more rational approach is required to exploit full therapeutic potential in this area. Current drugs have low potency and are relatively nonspecific, therefore there is a need for the development of subtype selective inhibitors, which might have greater efficacy with reduced side effects.^[5,6]

Recently, the first crystal structure of a Na_v channel from *Arco-bacter butzleri* was published.^[7] This crystal structure provides key insights into the molecular basis of electrical signaling, and provides a template for understanding the action of drugs at the atomic level. Structural information offers a good prospect for the development of efficient and selective Na_v modulators.

Alkaloids from the Caribbean sponge of the genus *Agelas*, e.g. monomers clathrocin and oroidin, and dimmers sceptrin and dibromosceptrin, have been shown to be active on muscle and nerve membrane receptors and channels, including Na_v. Studies suggest that clathrocin and dibromosceptrin affect Na_v channels by influencing channel ion conductance, and by modifying the channel inactivation characteristics, respectively.^[8]

We have designed and synthesized a series of oroidin analogs and evaluated their effects on several different Na_v channel subtypes. We have discovered that some of the compounds have promising activities on different Na_v subtypes, e.g. compound UL-NZ-10 modulates the activity of the Na_v 1.4 subtype by slowing down its inactivation. Na_v 1.4 ion channels are expressed mainly in the skeletal muscle, so compounds acting on those channels are expected to be potentially useful for the treatment of muscle disorders, such as hyperkalemic periodic paralysis and paramyotonia congenital. The availability of the first crystal structure of Na_v channel and some compounds with moderate subtype selectivity are good starting points for structure-based design of selective Na_v modulators.

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P055

Synthesis and Biological Evaluation of New Spiroisoxazoline Oxindoles as Potential Anticancer Agents

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The spiro-oxindole framework is present in several natural alkaloids and synthetic agents, which have shown important biological activity with potential use as, e.g., anti-inflammatory, antimalarial, and anticancer agents (Figure 1).^[1] Because of their remarkable biological activity, significant efforts have been devoted to the synthesis and evaluation of novel spiro-oxindole derivatives.



Figure 1. Spiro-oxindole derivatives with biological activity.

A small library of spiroisoxazoline oxindole compounds **1** were synthesized by reacting 3-methylene indolin-2-ones **2** with chloroamines **3** in the presence of triethylamine or zinc (Scheme 1).^[2] Their antiproliferative effects were investigated to monitor their potential antitumor activities. Cell viability was evaluated using a MTS assay in hepatocellular carcinoma Hep G2 cell line after exposure to the spiro-oxindole derivatives. Based on the information acquired from biological assays and structure–activity relationship studies, we are now synthesizing new spiro-oxindoles in order to obtain compounds with improved antiproliferative activity.



Scheme 1. Synthesis of spiroisoxazoline oxindoles.

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P056

Targeting Heme Detoxification Process for the Development of Structurally Diverse Antimalarials

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Malaria afflicts the populations of at least 102 countries, with about one billion people at risk of infection in tropical and subtropical areas. The history of malaria teaches that the parasite is extremely resourceful, and although in the last five years the research activity, capacity building and cooperation with endemic countries has been boosted by both USA and European authorities, the persistence of resistance and the limited number of therapeutic tools is compromising the way to elimination and then eradication of the disease. The continued emergence of drug-resistant parasites imposes an urgent need for a new generation of treatment and control measures. Our continuous effort in the field of drug discovery and development for malaria disease led to the identification of new classes of affordable, rapidly acting, and orally bioavailable drugs structurally based on novel pharmacophores with low potential to develop resistance.

P057

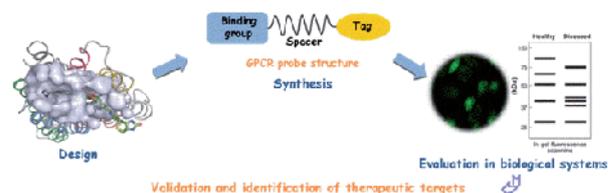
Development of Chemical Probes for the Study of G Protein-Coupled Receptors

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There is a significant decrease in the number of new drugs launched to the market, in spite of the efforts from both academia and industry. In order to solve this pharma innovation gap not only the discovery of new drugs is needed, but it is also crucial to validate/identify new therapeutic targets. In this context, activity-based protein profiling (ABPP) has emerged as a powerful chemical strategy to improve our knowledge of native biological systems. This approach has been successfully applied to the study of different enzyme families related to pathologies.^[1] However, no probes have been developed so far for the study of G protein-coupled receptors (GPCRs), which account for more than 50% of the druggable genome.^[2] In our project, we are involved in the development of chemical probes bearing fluorescent, photoactivatable and/or affinity tags aimed at visualization, isolation, enrichment and/or identification of GPCRs in complex biological systems.

Among the several hundreds of known GPCRs, we have focused our efforts on serotonin and cannabinoid receptors, due to their clinical significance and our previous experience.^[3] Our strategy encompasses the selection of adequate scaffold(s) targeting the receptor, the design of labeled ligands, the synthesis of the designed compounds, and the evaluation of their potential as chemical probes in biological systems of increasing complexity (see Figure).



Here, we will show our latest results focused on the serotonin 5-HT_{1A} and 5-HT₆ receptors,^[4] as well as in CB₁ and CB₂ cannabinoid receptors.^[5] Up to this moment, we have introduced different labelling moieties including fluorophores, biotin, benzophenone and terminal alkynes. Some of the synthesized probes display high affinity for the target receptors and have been used for their direct visualization in cell systems. In addition, dual probes that combine benzophenone and biotin or a fluorophore in the same molecule are being evaluated for covalent binding and affinity pull-down of target proteins. These strategies should contribute to optimize the therapeutic exploitation of known or new members of the GPCR superfamily by providing valuable information about their location or level of expression.

Acknowledgements: This work was supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and Comunidad Autónoma de Madrid (SAL-2010/BMD2353). The authors thank MINECO and European Social Fund for Juan de la Cierva, Ramón y Cajal, and FPU grants to J.A.G.-V., S.O.-G., and L.M.-C., respectively, and CAM for a predoctoral fellowship to A.M.G.

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P058

Design of Fusion Inhibitors of Flaviviruses

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Currently there is no effective therapy of flaviviral infections such as tick-borne encephalitis, dengue fever, West Nile fever, or yellow fever. Inhibition of viral fusion is a promising mechanism of action for new potential drugs against them. A hydrophobic pocket identified earlier^[1] in dengue virus envelope membrane-anchored protein E could accept fusion-preventing molecules. However, the fusion inhibition mechanism has not yet been studied in detail.

The constructed homology models of E proteins of several flaviviruses (DENV, TBEV, POWV) allowed us to perform virtual screening of available compounds by unguided docking into the aforementioned hydrophobic pocket. 12 of 100 compounds selected for experimental evaluation showed acceptable virus-growth inhibition, and two of them demonstrated low toxicity in vitro and in vivo tests.

For the molecular dynamics studies, we have constructed a homology model of the full building block of flaviviral envelope including stem and anchor parts of E protein and M protein based on the low resolution cryo-electron microscopy map.^[2] The model was preliminarily optimised using the implicit membrane model, and then the molecular dynamics simulation was performed with an explicit

membrane for both ligand-bound and ligand-free states of the system. Two protonation states were utilized for each conformation of the protein corresponding to fusion-inactive (neutral pH) and fusion-ready (low pH) states. A possible mechanism of inhibition was proposed.

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P059

Bis-Alkylamine Quindolone Derivatives: Structure–Antimalarial Activity Relationships

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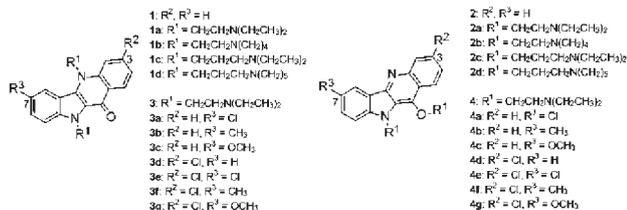
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Malaria is one of the most widespread infectious diseases of our time due to the rapid emergence and spread of multidrug-resistant strains of *Plasmodium falciparum*, the most lethal of the malaria parasite species.^[1] During their erythrocytic stage, malaria parasites feed on host hemoglobin releasing toxic free heme, which is biocrystallized into hemozoin or malaria pigment, harmless to the parasite.^[2] Heme detoxification remains one of the most attractive drug development targets mainly due to the immutable nature of the heme molecule. We previously showed that introduction of alkylamine side chains at indoloquinoline aromatic skeleton increase in vitro antiplasmodial activity and selectivity.^[3,4] We now report the antiplasmodial and cytotoxic activities of a 20 compound library of bis-alkylamine quindolone (indole[3,2-b]quinolin-11-one) derivatives designed to accumulate inside parasite acidic digestive vacuole and to bind to heme dimer and hemozoin crystal face {100}, which exposes twodimensional series of propionic acid anions.

Structure–antiplasmodial activity relationship analysis of side chain effects (**1a–d** and **2a–d**) reveal that: i) side chain length (2 or 3 C), ii) cyclic or linear alkyl substitution at terminal amine group, and iii) position of side chain (N^5, N^{10} -bisalkyl **1** or N^{10}, O^{11} -bisalkyl **2**) do not significantly influence the antiplasmodial activity. The effect on antiplasmodial activity of electron-withdrawing or electron-donating groups in position 7 of the quindolone skeleton, in the presence (**3d–g** and **4d–g**) or absence (**3a–c** and **4a–c**) of a

chlorine at position 3, was also investigated. The results show that two electron-withdrawing groups, such as chlorine, at positions 3 and 7 clearly induce a significant increase in antiplasmodial activity in the case of *N,O*-bisalkylamine substitution, but not in the case of *N,N*-bisalkylamine substitution, suggesting that electronic distribution at quinoline nitrogen could play an important role in antiplasmodial activity of bis-alkylamine quindolone derivatives. Overall, 3,7-dichloro *N,O*-bis-alkylated derivative **4e** emerges as the most active compound of the series, with an IC₅₀ value of 25 nM for the chloroquine-resistant *P. falciparum* W2 strain, and a selectivity ratio of approximately 10².

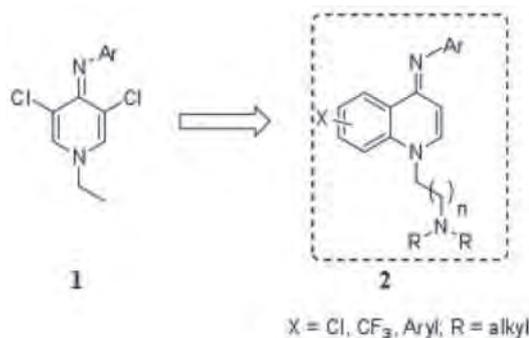


Acknowledgments: FCT (Portugal) for financial support (PTDC/SAU-FAR/114864/2009 and Pest-OE/SAU/UI4013/2011)

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Following our report on pyridonimines (**1**) as blood^[2]- and liver-stage active compounds,^[3] we now focused on the design and synthesis of a library of quinolin-4-imines (**2**) containing an alkylamine side chain at N-1 of the quinolinimine scaffold to improve aqueous solubility. Compounds **2** were synthesized in moderate to good yields, with the C=N bond in the *E*-configuration, as revealed by X-ray crystallography. Compounds **2** displayed excellent activity against the blood-stage of infection, with IC₅₀ values in the low nM region, and good activity against the liver-stage of infection, with IC₅₀ values in the low μM region. Although the mechanism of action for quinolin-4-imines **2** are not known, our results reveal that compounds **2** could offer starting points for the development of dual-stage antimalarial drugs.



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P060

N-(Alkylamine)-quinolin-4-imines as Novel Dual-Stage Antimalarial Compounds

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Malaria remains a major public health threat worldwide, with high mortality and morbidity burdens as well as serious economical and social impacts on the development of malaria-endemic countries. While drug-resistant malaria poses a continuous therapeutic challenge, no drugs targeting the symptomatic intraerythrocytic stage of infection have been introduced in the market over the past decade. In contrast with medicinal chemistry programs focusing on the erythrocytic stage of infection, the liver stage of infection is underexploited and presents an opportunity to successfully develop new drugs.^[1]

P061

Pharmacophore-Based Drug Design for Casein Kinase 1 in Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cortex atrophy and loss of cortical and subcortical neurons. Recent data indicates the presence of Casein kinase 1 isoforms (CK1δ and CK1ε) in vacuolar strands and granulomatous lesions in AD patients' brain. CK1 is an attractive therapeutic target since it does not present the usual side effects caused by other pro-

teins, whereas the inactivation of one protein triggers the inhibition of several essential enzymes.^[1] This protein encompasses a large family of monomeric serine/threonine protein kinases found in a variety of subcellular locations.^[2] The first tridimensional structure was solved by X-ray crystallography in 1995,^[3] and nowadays 16 structures are deposited in the Protein Data Bank^[4] (PDB). In this work, search was performed in the BindingDB^[5] database for CK1 inhibitors, and the best compounds are been analyzed in the CK1 binding site.

Multiple global alignment was performed with the software UGENE^[6] using available crystallographic complexes of CK1 in PDB to analyze the similarity of these isoforms. In addition, the structural similarity was investigated by protein superposition, using SwissPdbViewer^[7] and Discovery Studio.^[8] A human δ isoform (PDB code: 3UYT/3UZP) related to AD was chosen to analyze the results obtained with previous pharmacophore model experiments.^[9]

Different pharmacophore models were derived with the PharmaGist^[10] and Discovery Studio using 4 CK1 δ inhibitors (PDB code: 1CKJ, 1EH4, 2CSN, 3UYT/3UZP) of crystallographic complexes. The best model obtained in consensus was used for pharmacophore-based virtual screening experiments with the Discovery Studio package and the ChemBridge^[11] and ZINC^[12] databases. The best-ranked 100 compounds of each database are being analyzed within the CK1 active site using the δ isoform (PDB code: 3UYT/3UZP). In addition, another ligand-based drug design method has been employed, which is based on 2D-similarity of the active compounds. Several novel compounds have been thus selected with significant Tanimoto index, which could be promising CK1 inhibitor candidates for future Alzheimer's disease treatment.

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P062

Synthesis and Pharmacochemical Study of Some 1-Acyl-2-pyrrolidones and Related Compounds

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Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system, and there are many subtypes of GABA receptors, which may explain why GABA deficiency is associated with many disease states, such as anxiety, convulsions, Parkinson's disease and central pain. GABA receptors have also been identified at the hypothalamic and pituitary levels and seem to play a role in the inhibition of hypothalamic-pituitary-adrenocortical axis. Thus, the synthesis of effective GABA agonists is challenging. In addition, cyclised GABA derivatives such as piracetam and aniracetam, apart from their anxiolytic activity, can modulate AMPA receptors, demonstrating nootropic-neuroprotective activity. It is known that seizures can generate brain oxidative stress. Oxidative insult is considered to be a mechanism playing an important role in the aetiology of seizure-induced neuronal death. Furthermore, cyclooxygenase-2 (COX-2) expression has been found increased in cells under kainic acid stress, and treatment with GABA reduced significantly COX-2 and prostaglandin E₂ production.

In this paper, we report the synthesis of the open-chain amides of GABA with trolox ((*R*)-3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylic acid), 3,5-di-*tert*-butyl-4-hydroxybenzoic acid and lipoic acid (5-(1,2-dithiolan-3-yl)pentanoic acid), their ethyl esters and their cyclisation to *N*-acyl-2-pyrrolidones. These compounds may be of wider biological interest, since they could be GABA prodrugs, possible nootropics, aniracetam-related structures. The antioxidant activity of these compounds, their ability to inhibit COX-1, COX-2 and lipoxygenase, as well as their effect on acute inflammation are investigated. We calculated lipophilicity and topological polar surface area of these molecules, since these physicochemical properties are crucial for membrane penetration.

The synthesis of GABA amides is conducted by conventional methods using trimethylsilyl esters of GABA. Their cyclisation to *N*-acylpyrrolidin-2-ones is achieved using CDI.

Their effect on microsomal membrane lipid peroxidation was examined. It is found that, in all cases, the formation of the pyrrolidinone structure contributes to a large increase of antioxidant activity. These results cannot be entirely attributed to physicochemical properties, i.e. lipophilicity and polar area, although lipophilicity in general is an important factor for compounds acting as inhibitors of lipid peroxidation. Most of the examined compounds inhibit cyclooxygenase and lipoxygenase in vitro and reduce acute inflammation by more than 40%.

It can be concluded that amides of antioxidant acids with GABA in an open and, especially, in a cyclised, 2-pyrrolidinone structure are promising lead compounds for degenerative conditions.

P063

Biological Evaluation of New Bisacylimidoselenocarbamates as Potential Antiproliferative Agents in Cancer Treatment

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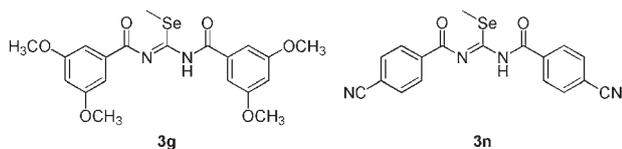
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Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008.^[1] It's well known that the trace element selenium (Se) appears to have cancer preventive properties based on a converging body of evidence from epidemiologic, clinical and experimental studies.^[2,3] Although the mode of anticancer action of Se is not fully understood yet, several mechanisms, such as antioxidant protection by selenoenzymes, specific inhibition of tumor cell growth by Se metabolites, modulation of cell cycle and apoptosis, and effect on DNA repair have all been proposed.^[4]

Among the growing list of seleno-compounds with desirable anticancer activity, we previously reported the synthesis of various bisacylimidoselenocarbamates with significant in vitro antiproliferative activity against human prostate cancer cells PC-3.^[5] To further characterize the antitumour activity of these compounds, here we extend the evaluation of the antiproliferative action of two of them, compounds **3g** and **3n**, to a panel of four human cancer cell lines (CCRF-CEM, HTB-54, HT-29 and MCF-7) and one non-malignant cell line (184B5). We also analyze the ability of **3g** and **3n** to induce apoptosis in CCRF-CEM and MCF-7 cells, as well as their effect on mitochondrial events in MCF-7 cells.



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P064

Synthesis of Novel Janus Kinase Inhibitors via Copper-Catalyzed Azide–Alkyne Cycloaddition

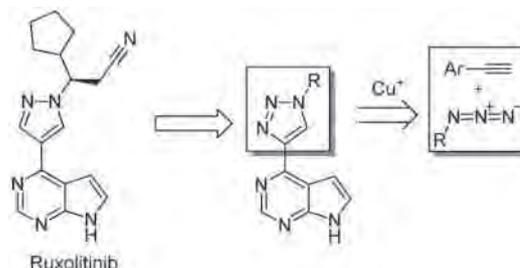
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Janus kinases (JAKs) are non-receptor protein tyrosine kinases mediating signaling through the JAK-STAT (signal transducer and activator of transcription) pathway. The Janus kinase family has four members: JAK1,2,3 and TYK2. Being crucial signal transducers for a variety of cytokines, growth factors, and interferons, JAKs are involved in numerous pathologies including malignancies, myeloproliferative disorders and autoimmune diseases.

In contrast to the ubiquitous expression of the other JAK family members, JAK3 is predominantly expressed in hematopoietic cells. In mammals, the lack of functional JAK3 causes immunodeficiencies while not disrupting the function of non-immune cells. Therefore, targeting JAK3 is a promising strategy to generate a novel class of immunosuppressant drugs with limited side effects.^[1]

Recently, Ruxolitinib, a small-molecule JAK1/2 inhibitor, was approved by the US Food and Drug Administration (FDA) for the treatment of patients with intermediate or high-risk myelofibrosis.^[2]



In search for novel JAK3 inhibitors, we replaced the Ruxolitinib pyrazole ring by a 1,4-disubstituted 1,2,3-triazole accessible through copper-catalyzed azide–alkyne cycloaddition.^[3] Compared to the laborious synthesis of the corresponding pyrazoles, click chemistry offers rapid and efficient access to triazoles with various substitution patterns facilitating their optimization towards JAK3 inhibition.

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P065

Organometallic Selective Estrogen Receptor Modulators—A Computational Approach to the Role of the Metal Moiety

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Primary treatment for prostate and breast cancer has been focused on hormone therapy, where selective estrogen receptor modulators (SERM) have played an important role especially in breast cancer treatment and chemoprevention. Raloxifene is an estrogen receptor α (ER α) antagonist that acts by binding the receptor and blocking its activation, thus inhibiting the growth of estrogen-dependent cancer cells, similarly to tamoxifen (the first developed ER α antagonist) but with a lower incidence of uterine cancer risk in treated patients. Based on raloxifene, various organometallic drug-like compounds have been developed by designing molecules with a metallocene moiety attached to the benzo[*b*]thiophene moiety of raloxifene. By maintaining a part of the raloxifene skeleton, these drugs are also able to antagonize the ER α . In addition, they have been shown to be cytotoxic in various tumor cell lines, and that has been attributed to the presence of the metallocene and its potential role in the generation of an oxidative environment.^[1]

Using a computational approach, we studied the interaction of some metallocene-containing benzo[*b*]thiophenes with ER α by employing protein–ligand docking techniques. We also computed their oxidation potentials as a first approach to study their role in oxidative stress.

The docking results obtained indicate that the metallic moiety does not contribute to the ligand–protein interaction, as it rests outside the protein, and the binding affinities are roughly independent of the type of metallocene considered. On the other hand, changing the ligand part does not significantly affect the vertical ionization potential, indicating that these compounds retain most of the oxidation–reduction properties of the isolated metallocene.

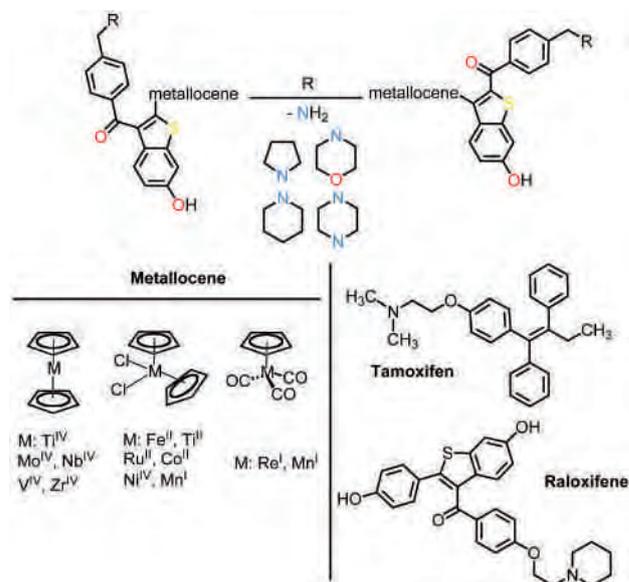


Figure 1. Structure of the combinatorial library prepared. The basic structure of the metallocenyl-containing benzo[*b*]thiophenes studied is depicted together with the structures of tamoxifen and raloxifene.

Acknowledgments: This work was supported by the FCT (PTDC/QUI/67522/2006, PEst-OE/QUI/UI0100/2011, SFRH/BD/80690/2011 and SFRH/BD/80690/2011).

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P066

Synthesis of Novel Hybrid Coumarin and Bis-Coumarin Derivatives as Lipoyxygenase Inhibitors and their Potential Role as Anticancer Agents

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Although anti-inflammatory drugs are used extensively, prolonged consumption of these medications is usually coupled with numerous side effects. Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediators with the help of natural dietary products. Coumarins are naturally occurring benzopyrene derivatives found in a variety of plant sources.

The biological properties and the therapeutic applications of simple coumarins depend upon the pattern of their substitution. Chalcones or 1,3-diaryl-2-propen-1-ones are open analogues of flavonoids in which the two aromatic rings are connected by a three-carbon α,β -unsaturated carbonyl system. Each group of compounds was found to possess antioxidant, antibacterial, antiviral and antifungal activities. A lot of reports describing their anticancer and anti-inflammatory properties have been published.^[1,2]

Leukotrienes are bioactive lipid mediators involved in inflammation, allergy, cardiovascular diseases and cancer. Lipoxygenase (LOX) is the key enzyme in leukotriene biosynthesis catalyzing the initial transformation of arachidonic acid. Thus LOX is a suitable drug target for inflammation as well as cancer treatment and prevention.^[3]

Structure-based virtual screening performed on more than 250 coumarin derivatives, comprising hybrids molecules of coumarin-chalcones, led to the identification of novel LOX inhibitors. Other derivatives were also designed by the means of a previously derived QSAR model of anticancer chalcones.^[4] Chalcones were developed through a base-catalysed Claisen-Schmidt condensation reaction between the appropriate substituted acetophenone and aldehyde. The corresponding chalcone is conjugated with 4-hydroxy-coumarin, following a Michael catalyzed reaction, giving the desired hybrid product. In order to delineate the role of the structural characteristics upon the biological responses, 4-hydroxy-coumarins reacted with the appropriate aldehyde in a 2:1 ratio resulted to the respective bis-4-hydroxy-coumarin derivatives. The compounds have been identified using IR, ¹H NMR, ¹³C NMR, elemental analyses and mass spectroscopy.

Compounds have been tested for their ability to inhibit in vitro soybean lipoxygenase. Furthermore, the title compounds were evaluated for their antiproliferative activity in different cancer cell lines (US National Cancer Institute). The results are discussed in terms of structural characteristics and physicochemical properties.

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P067

Novel Selenocarbamates as Antiproliferative and Antileishmanial Agents

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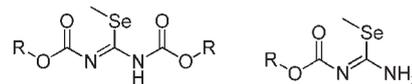
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Cancer is still a major health problem, being the second most common cause of death worldwide.^[1] The serious problems still associated with the treatment point out the urgent need to search for novel, more efficient and safe chemotherapeutic agents. Moreover, several of the most effective antiprotozoal agents were originally developed as anticancer drugs,^[2] which encouraged us to search for antileishmanial agents also. Leishmaniasis is still one of the world's most neglected diseases, affecting largely the poorest of the poor, mainly in developing countries.^[1] This disease is caused by several species of *Leishmania* protozoa in the Trypanosomatidae family.^[3]

Our main focus in the laboratory is the synthesis of selenium (Se)-containing compounds. Se is an essential dietary component of fundamental importance to human health. More than 200 studies support the anticarcinogenesis effects of Se, and several mechanisms have been suggested; the major ones are reduction of DNA damage, oxidative stress, inhibition of cell cycle and angiogenesis and induction of apoptosis.^[4] There are also several reports that have shown the role of selenium in the modulation of the immune response against *Trypanosoma* infections.^[3]

This study aims at the synthesis of a series of new selenocarbamates with structures **1** and the evaluation of their antitumoral and antileishmanial activity in vitro.



1: R=heterocyclic, aliphatic, aromatic groups

Antitumoral evaluation has been carried out in vitro against prostate cancer cell line (PC3), and cytotoxic parameters (GI_{50} , TGI and LD_{50}) have been determined. The GI_{50} values for seven of the compounds were below $1 \mu M$, lower than some standard chemotherapeutic drugs used as references. Antileishmanial activity has been evaluated against amastigotes, and the selectivity index (SI) was defined using a leukemia cell line derived from monocites (THP-1). We have also seen that our compounds showed potent antileishmanial activities.

Acknowledgements: B. Romano acknowledges the Association of Friends of the University of Navarra for a Ph.D. grant and project funding by the Spanish Ministerio de Ciencia e Innovación.

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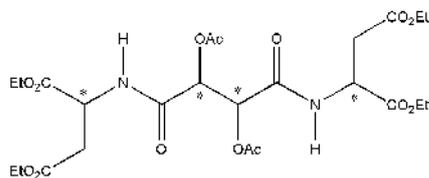
P068

Evaluation of the Stereoselectivity of Binding of Tartaric Acid Pseudopeptides at the Active Site of HCV Protease

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Hepatitis C is an inflammatory disease of the liver caused by infection with hepatitis C virus (HCV), affecting 3% of world population according to the World Health Organization (WHO). Currently, the treatment of hepatitis C uses α -interferon and ribavirin, which is not only expensive, but also suffers from disadvantages such as varied effectiveness in relation to the HCV genotypes, severe side effects and the need of intravenous use. Due to the disadvantages of current treatment, new strategies for anti-HCV therapy are under development.^[1] One of the most promising strategies is based on the inhibition of HCV protease, which is crucial for the production of components related to the virus replication. The present work aims the synthesis and pharmacological evaluation of pseudopeptides derived from L-, D- or meso-tartaric acid and from L- and D-aspartic acid as inhibitors of HCV protease. The general structure of these prototype protease inhibitors is depicted below.



For the developing process of specific HCV serine protease inhibitors, we proposed, based on the literature models,^[2] the inactivation of the catalytic triad by forming a stable acyl–enzyme complex. Thus, these substances must have a group capable of transferring an

acyl residue, which is present in the acetyloxy ethylene core. Seven stereo isomers of the studied pseudopeptide were synthesized, and the results obtained in the HCV protease inhibition tests are shown in Table 1.

Table 1. Inhibition of the proteolytic activity of HCV protease by tartaric acid pseudopeptides.

Compd [100 μ M]	Abs. configuration	Rel. activity ^[a] [%]
control	x	100
1a	(S,R,R,S)	100
1b	(S,S,S,S)	100
1c	(S,S,R,S)	110
1d	(R,R,R,R)	110
1e	(R,S,S,R)	63
1f	(R,S,R,R)	88
1g	(S,R,R,R)	102

[a] Relative activity of HCV protease.

The results obtained show that there is a unique stereochemical pattern recognized by this enzyme, which is the one present in compound **1e**, suggesting a highly stereospecific interaction between the enzyme and the pseudopeptides tested.

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P069

Structure-Based Design and Synthesis of Inhibitors for Arylsulfate Sulfotransferase: New Antibiotics against Urinary Tract Infections

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The crystal structure of the PAPS-independent arylsulfate sulfotransferase (ASST) was solved in 2008 after isolation from uropathogenic *Escherichia coli*. ASST catalyzes the transfer of a sulfonyl group from an activated donor to an acceptor.^[1] In doing so, many physiological processes can be initiated and carried out, for example, the detoxification of medically active compounds. Since uropathogenic *E. coli* (UPEC) can cause urinary tract infections (UTIs), ASST represents an attractive target for the treatment of UTIs. We are thus trying to inhibit the highly polar binding site of ASST in order to develop new antibiotics and to gain insight into the binding mode, by investigating the various interactions of the synthesized inhibitors with the active site of ASST.



Figure 1. Dimer of ASST which catalyzes the PAPS-independent transfer of sulfuryl groups within UPEC.

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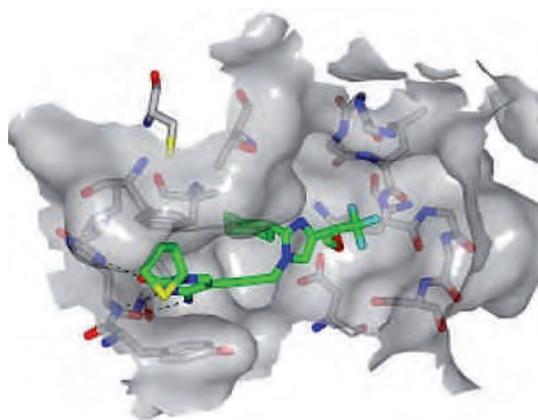
P070

Rational Design, Synthesis and Biological Testing of Inhibitors for IspE

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The kinase IspE is involved in the mevalonate-independent biosynthetic pathway of isoprenoid precursor synthesis. The pathway is used exclusively by many pathogens (e.g. *Plasmodium falciparum* and *Mycobacterium tuberculosis*) but not by humans. This fact makes the enzymes of the mevalonate-independent pathway promising targets for the development of new drugs against malaria and tuberculosis.

Our research group reported the development of active inhibitors, displaying inhibitory constants (K_i) in the nanomolar range against the enzyme from *Escherichia coli*.^[1] A rational, structure-based design approach was employed for a new class of compounds as potential inhibitors for IspE. The synthesis and biological evaluation of the new ligands are presented.

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P071

Optimisation of Jasmonic Acid Structure for New Topical, Skin Antiaging Application

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Jasmonic acid is implied in wound and defense-signalling pathways of plants. Surprisingly, we were able to prove that in vitro treatment of stratum corneum with jasmonic acid resulted in corneocyte desquamation—a result generally observed with salicylic acid. To optimize these exfoliating properties essential in the cosmetic and dermatologic treatment of desquamation disorders like those occurring during aging and/or during the winter season, we prepared jasmonic acid derivatives. The goal was to identify by a structure–activity relationship (SAR) study the most efficient analogue. The tetrahydrojasmonic acid gave us the best activity in a simple in vitro stratum corneum desquamation model, which estimates the number of corneocytes released. Moreover, this property was confirmed in a more elaborated model using a reconstructed epidermis. This effectiveness indicates this molecule as a new and promising candidate for the treatment of desquamation disorders and to improve the signs of the skin aging.

P072

Novel Sulfonamides: A New Class of Potent Antileishmanial Agents

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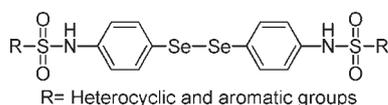
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Leishmaniasis is an ancient protozoan disease affecting about 12 million people with 2 million new cases every year that constitute a serious public health problem. According to the World Health Organization (WHO), leishmaniasis is now endemic in 88 countries, particularly in subtropical and tropical regions.^[1]

Selenium is a prominent trace element, whose increased concentration in plasma has been recognized as a new defensive strategy against *Leishmania* infection^[2,3] and after the work developed by our research group,^[4,5] we realize that diselenide group is important to achieve potential compounds. Besides, sulfonamide compounds present antiparasitic activity including an antileishmanial profile.

We carried out the synthesis and biological evaluation of new sulfonamide derivatives, according with this general structure:



All the synthesized compounds were subjected to in vitro screening against *L. infantum* amastigote model. In order to establish the selectivity index (SI), their cytotoxic effect was carried out against Jurkat and THP-1 cell lines.

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P073

PDE Inhibitors as Potential Treatment of African Sleeping Sickness—A New Disease for an Old Target or a New Target for an Old Disease

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African sleeping sickness causes significant morbidity and mortality and seriously affects society in the poorest areas of the world. The current therapies suffer from severe side effects, inconvenient administration and high costs.^[1]

In the search for an urgently needed new treatment, cyclic nucleotide phosphodiesterases (PDEs) have emerged as attractive molecular targets. For example, both genetic knock-down and chemical inhibition of PDE activity resulted in halted proliferation and eventually elimination of *Trypanosoma brucei* (Tbr), the causative agent of African sleeping sickness.^[2]

The vast knowledge and generated expertise within the field of human PDEs has provided a shortcut to low-affinity inhibitors of parasitic PDEs. Scarcity in the drug research pipeline can thus be compensated with better pharmacological predictability and profound understanding of possible adverse effects. Interestingly, TbrPDEB1 and TbrPDEB2 are cAMP specific, like one of the most investigated human PDEs, hPDE4. The catalytic domains of the hPDE4 and parasitic PDEs show a high degree of homology, as well as parasite-specific features (Figure 1).^[3] This has allowed fast optimization of hit compounds and generated nanomolar TbrPDE inhibitors with trypanocidal activity.

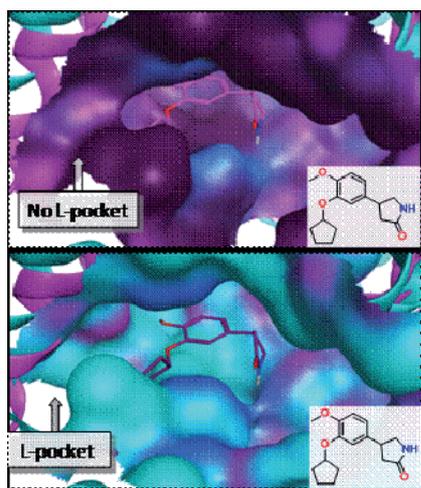


Figure 1. Top) The vdW surface of the active site of hPDE4B (magenta) co-crystallized with rolipram; Bottom) The homology model TbrPDEB1 (cyan) with rolipram superimposed and the arrow pointing at the parasite-specific P-pocket.

Acknowledgements: This project is facilitated by Dutch Top Institute Pharma and involves eight consortium members and research labs in four countries.

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P074

Probing the Bicyclic Hydroxypyrazolo[1,5-*a*]pyridine Scaffold as a Carboxylic Acid Bioisostere in the GABA_a Receptor System

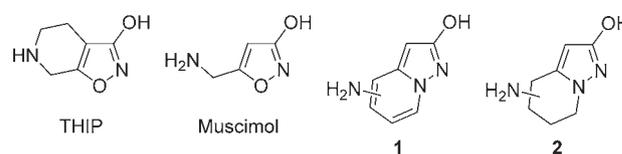
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Isosteric replacement is a widely used approach within medicinal chemistry for improving properties of a lead compound such as bioavailability, selectivity, and potency. A number of bioisosteric relationships have been established for a number of functional groups including the carboxylic acid. Heterocycles such as tetrazole, 3-hydroxyisoxazole, 3-hydroxyisothiazole, 3-hydroxy-1,2,5-thiadiazole, 3-cyclobutene-1,2-dione and the 1,2,5-oxadiazole system have been successfully applied as carboxylic acid bioisosteres. Medicinal chemistry programmes have provided an extensive variety of bioisosteric replacements for the carboxylic acid in GABA, the major inhibitory neurotransmitter in the mammalian central nervous system. The

3-hydroxypyrazole ring system has previously been shown to be a bioisostere of the carboxylic acid of GABA within the GABA_a receptor system. In this study, we introduce the bicyclic hydroxypyrazolo[1,5-*a*]pyridine scaffold (see scaffold **1**) as the main backbone of potential ligands for the orthosteric site in the GABA_a receptor. Apart from mimicking the acidic properties of the carboxylic acid group in GABA and the 3-hydroxyisoxazole in the GABA_a agonists, THIP and muscimol, the conformationally locked hydroxypyrazolo[1,5-*a*]pyridine moiety offer additional positions for introducing substituents in fixed directions. Taking advantage of this option, we have investigated the effect of introducing the amino containing substituents in different positions of the scaffold (**1**) and of the corresponding piperidine scaffold (**2**), thus enabling investigation of the requirement for the mutual position of the functional groups and exposing access to the cavities/channels associated to the orthosteric binding site, reaching out for subtype-selectivity.



A series of hydroxypyrazolo[1,5-*a*]pyridine (**1**) and hydroxypyrazolo[1,5-*a*]piperidine (**2**) derivatives were synthesized and pharmacologically characterized in a [³H]muscimol displacement assay at native GABA_a receptors and electrophysiological assays at relevant GABA_a receptor subtypes. The synthesis and pharmacological properties are reported and discussed in terms of the structural knowledge available for the GABA_a receptor.

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P075

Discovery of a Novel Class of Reversible Monoamine Oxidase B Inhibitors Based on Chromone Scaffold

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Monoamine oxidases (MAOs) are widely distributed enzymes that contain a flavin adenine dinucleotide (FAD) unit covalently bounded to a cysteine residue.^[1] Many living organisms possess MAOs and in mammals two isoforms are present, MAO-A and MAO-B, which are located in the outer membrane of the mitochondria. The MAO-B isoform has a crucial role in neurotransmitter metabolism, representing an attractive drug target for neurodegenerative diseases therapy, such as Parkinson's disease (PD). PD is a neurodegenerative disorder characterized by a myriad of symptoms that gradually decrease the life quality of the patient. At present, monoamine oxidase inhibitors (IMAO), specifically of MAO-B type, are considered also to be beneficial therapeutic drugs. The inadequacy of the current pharmacotherapy and the lack of drugs that can be effective in PD, mainly declined by side-effects, are the reasons why the discovery of novel chemical entities (NCE) is still a demand.

Chromones (benzo- γ -pyrone) are one of the most abundant groups of naturally occurring heterocyclic compounds. Because of their structural features they are important building blocks in the natural product and synthetic organic chemistry areas. In addition, remarkable antioxidant, anticancer and enzymatic inhibition activities were ascribed to these benzopyrone compounds.

The present project consists on the design and development of a versatile library incorporating a privileged structure based on the benzo- γ -pyrone scaffold as a putative shortcut for the early drug-development stage on the discovery of new NCE for IMAO-B. Accordingly, a diversity-oriented synthesis methodology was adopted by means of modular syntheses that involve few steps, to obtain structurally varied drug-like compounds. Efforts were done to cover as much chemical space as possible to maximize the likelihood of discovering a novel and patentable lead class of active compounds. The results obtained so far will be presented supported by synthetic, biologic and docking studies, pointed out a crucial and undisclosed role of the presence of a carboxamide group in C3 of the pyrone ring that is able to establish hydrogen bond interactions with the active site of the MAO-B enzyme.^[2]

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P076

Thiazole-Substituted Pirinixic Acid Derivatives as Dual 5-LO/mPGES-1 Inhibitors

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Imbalances in the lipid signaling network contribute to the pathogenesis of a large number of human diseases. The metabolic fate of arachidonic acid (AA) plays a crucial role within this network and is associated with pathophysiological conditions such as inflammation, analgesia, asthma and cancer.

The metabolic pathway of AA can be divided into two different ways: The formation of prostaglandins (PGs) by cyclooxygenases (COXs) and the biosynthesis of leukotrienes (LTs) by 5-lipoxygenase (5-LO). Nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs) are the most wide-spread drugs in the anti-inflammatory therapy. However, especially in long-term therapy their use is closely related to severe side effects such as gastrointestinal and renal complications (NSAIDs) or an increased cardiovascular risk (coxibs) due to the suppression of physiological relevant prostaglandins.^[1] Consequently, new pharmacological strategies for anti-inflammatory therapy are urgently needed. One promising approach is the selective inhibition of downstream-acting enzymes such as the microsomal prostaglandin E₂ synthase-1 (mPGES-1), which catalyzes the formation of PGE₂ from PGH₂. PGE₂ is the most prominent mediator in inflammatory pain. On the other hand, LTs produced by 5-LO are important inflammatory mediators which act as bronchoconstrictors and increase vascular permeability. The dual inhibition of 5-LO and mPGES-1 is considered as a novel strategy to avoid COX-related side effects such as the analgesic asthma syndrome and to maintain the physiological prostaglandin levels.

The structural basis of the presented compounds is pirinixic acid, which is inactive on both, mPGES-1 and 5-LO. Especially the introduction of *n*-alkyl chains in α -position led to potent dual 5-LO/mPGES-1 inhibitors. Furthermore, a broad modification of the lipophilic backbone is possible with an equal or increased activity.^[2] Herein, we present a novel class of pirinixic acid derivatives featuring a thiazole scaffold at the lipophilic backbone. The resulting thiazole substituted derivatives show balanced dual inhibition of mPGES-1 and 5-LO with IC₅₀ values from the high nM to low μ M range.

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P077

The Antiepileptic Drug Carbamazepine: Blood Protein Adducts as Possible Biomarkers of Toxicity

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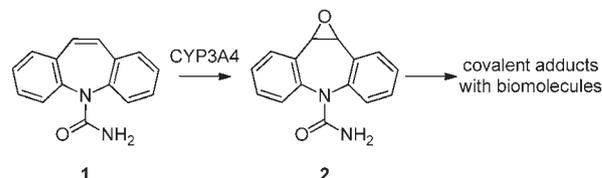
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Epilepsy is defined as a brain disorder characterized by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures. This chronic neurological disease affects about 50 million people of all ages worldwide.^[1] Aromatic antiepileptic drugs (AAEDs) are used in long-term treatment of epilepsy, chronic pain and psychiatric diseases (e.g., bipolar disorders, anxiety disorders, and schizophrenia).^[2] Despite their widespread use, AAEDs are related with serious idiosyncratic drug reactions (e.g., skin reactions, multiorgan hypersensitivity, immune-mediated hypersensitivity, and hepatotoxicity), which can be life-threatening.^[3] Although the mechanisms that explain these side effects are currently not clear, the involvement of reactive metabolites capable of binding with biomolecules has been hypothesized.^[4]

Carbamazepine (CBZ, **1**) is one of the most widely used AAEDs for both adults and children. However, its association with central nervous system toxic events and hypersensitivity reactions raises concerns about its chronic administration. CBZ undergoes cytochrome P450 3A4-mediated epoxidation with the formation of its major metabolite, carbamazepine-10,11-epoxide (CBZE, **2**).^[5] This reactive metabolite can undergo ring opening reactions in the presence of bionucleophiles (e.g., proteins) yielding covalent adducts that may be at the genesis of the toxicity outcomes linked with the parent drug. Reactive metabolites are short-lived species in vivo, a characteristic that makes them extremely difficult to detect; consequently, the establishment of direct correlations between metabolite levels and the induction of specific pathologies is not straightforward. However, the stable covalent adducts formed with the easily accessed blood proteins, human serum albumin (HSA) and hemoglobin (Hb),

have been extensively investigated as biomarkers of exposure to toxicants, in search of potential dose–toxicity correlations enabling the establishment of risk–benefit relationships.

We synthesized CBZE and investigated its reactivity towards nucleophilic amino acids (e.g., *N*-acetyl-L-cysteine, ethyl L-valinate) and human blood proteins (Hb and HSA). We obtained evidence of covalent binding to the bionucleophiles. These results support a role for CBZ bioactivation at the onset of the toxic effects elicited by this antiepileptic drug and suggest that covalent adducts formed with blood proteins can be used as biomarkers of CBZ toxicity.



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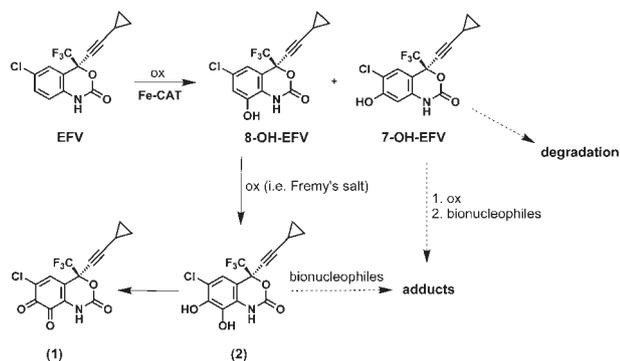
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P078

Oxidized Derivatives of Phenolic Metabolites from the Anti-HIV Drug Efavirenz – A Plausible Role in Toxicity

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Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) administered as first-line treatment against HIV. Despite its efficacy, EFV use is associated with neurotoxic and hepatotoxic events.^[1] There is evidence that metabolic activation of EFV to reactive electrophiles (i.e., catechols and quinone species) capable of reacting with bionucleophiles leads to the formation of covalent adducts that are involved in the initiation of species-specific toxic outcomes. Thus, elucidation of the reactivity of EFV metabolites and their oxidized derivatives is of great interest to assess the role of these adducts in the origin of toxic events. We synthesized two major metabolites of EFV, 8-hydroxy-efavirenz (8-OH-EFV) and 7-hydroxy-efavirenz (7-OH-EFV), both using a conventional synthetic strategy^[2] and a new “bio-inspired” catalysis, directly from EFV.^[3] The latter method, using a Fe(II) catalyst, provides the enantiomerically pure metabolites and mimics the cytochrome P450-mediated oxidation mechanisms.

Based on our experience with another NNRTI, nevirapine,^[4] we further explored the chemical oxidation of 8-OH-EFV and 7-OH-EFV with Frémy's salt. For the first time, we detected the formation of a quinone intermediate (1) and its catechol precursor (2) from 8-OH-EFV. By contrast, the direct oxidation of 7-OH-EFV under analogous conditions does not appear not to pass through the expected *ortho*-quinone (1). Nonetheless, the oxidized species demonstrated, in both instances, an ability to react with model bionucleophiles (e.g., ethyl valinate, *N*-acetylcysteine) yielding covalent adducts. These results support a role for oxidized derivatives of phenolic EFV metabolites at the onset of EFV-mediated toxicity.

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P079

Structural Modifications of NSAIDs Targeting Inflammation, Dyslipidemia and Safe Profile

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The great majority of nonsteroidal antiinflammatory drugs (NSAIDs) act via inhibition of cyclooxygenase, thus preventing prostaglandin biosynthesis. However, this mechanism of action is also responsible for their main undesirable effect, gastrointestinal ulceration. Furthermore, it is well established that reactive oxygen species play a decisive role in inflammatory conditions. It has also been noted that some antioxidant compounds exhibit antiinflammatory activity, while oxidative stress is an important component of toxicity. Thus, the discovery of molecules, which combine anti-inflammatory and antioxidant activities may lead to the development of drugs with an improved therapeutic index. In this respect, the chemical derivatization of known NSAID molecules to incorporate antioxidant properties may be a useful approach, provided that the molecular modifications do not abolish the antiinflammatory activity. It has been shown that ester and amide derivatives of NSAIDs are potent cyclooxygenase-2 inhibitors and retain the anti-inflammatory activity of the parent NSAIDs. It is also accepted that atherosclerosis is a chronic inflammatory response. A number of known NSAIDs present a good anti-dyslipidemic action. We have demonstrated that antioxidant properties of novel anti-dyslipidemic compounds are beneficial for their action.

In this communication, we report the design, synthesis and pharmacological evaluation of amide derivatives of well-established NSAIDs with L-cysteine ethyl ester. Due to the presence of the SH functional group, the latter moiety is likely to confer antioxidant and cytoprotective properties to the novel compounds. Further antioxidant properties are expected to be offered by esterification with salicylic alcohol, butylated hydroxybenzyl alcohol and quercetin.

The synthesized compounds were evaluated for anti-inflammatory (carrageenan-induced paw oedema model), anti-dyslipidemic (in hyperlipidemic rats), antioxidant (inhibition of lipid peroxidation) activities, as well as for their effect on cyclooxygenases and lipoxygenase. Gastrointestinal toxicity and hepatoprotective action were estimated for selected structures.

It is found that the novel compounds, as designed, acquired all the desired properties, that is, in vivo and in vitro anti-inflammatory, antioxidant and considerable anti-dyslipidemic action.

In conclusion, the conjugation of the carboxylic group of known NSAIDs with antioxidant molecules is well tolerated and results in compounds with considerable anti-inflammatory activity. Furthermore, this molecular modification confers to the molecules antioxidant activity, while it also reduces their GI toxicity and conveys cytoprotection. This kind of chemical derivatization of NSAIDs may offer a viable route to safer anti-inflammatory agents which, having additional beneficial properties such as antidyslipidemic activity, may comprise useful candidates for long term administration in conditions involving chronic inflammation.

P080

Design, Synthesis and Biological Evaluation of Enone Derivatives

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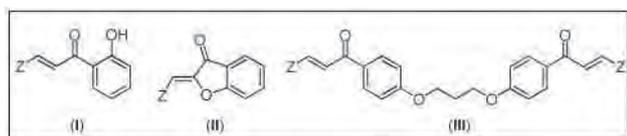
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Chalcones are enone derivatives, which are abundant in edible plants. They are precursors of flavonoids and many other biologically active molecules, such as aurones. Chalcones display a wide variety of biological activities including anti-inflammatory, antioxidant, antibacterial, anticancer, antiangiogenic, antimalarial, and antileishmanial activities. The same biological activities were reported for other enone derivatives, such as bis-substituted chalcone ethers.^[1,4]

Aurones are rarely occurring in nature, and they are biosynthesized from chalcones by the enzyme auresidin synthetase. The existing data on the bioactivity of natural and synthetic aurones are very promising, thus these heterocyclic compounds can be considered as an attractive scaffold for drug design and development. Aurones have been reported to possess insect antifeedant activities, anticancer, antileishmanial, anti-inflammatory and antibacterial properties. In nature, they are found in the flowering parts of many plants, and they are named after their bright yellow color.

Using computer-aided drug design and previous biological data from known chalcones and aurones, we designed a series of a) chalcones, b) aurones and c) bis-substituted chalcone ethers with possible inhibition on lipoxygenase, anticancer and anti-inflammatory activities in vivo.^[2,3,4]



A) 2'-Hydroxy-chalcones (I) were synthesized via the Claisen-Schmidt condensation reaction between 2'-hydroxy-acetophenones and appropriately substituted aromatic aldehydes in basic conditions; B) the synthesis of the desired aurones (II) includes an oxidative cyclization using mercury(II) acetate in pyridine; C) after the etherification of 4'-hydroxy-acetophenones and via the Claisen-Schmidt condensation with suitable substituted aldehydes the bis-substituted chalcone ethers were derived.^[1,3,4] The structures of the synthesized compounds were confirmed spectroscopically and by elemental analysis.

The compounds were tested in vitro for their ability to: a) scavenge the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical in different concentrations, b) inhibit lipid peroxidation of linoleic acid, c) inhibit in vitro soybean lipoxygenase, d) interact with glutathione, e) inhibit in vivo carrageenin-induced rat's paw edema and f) act as a toxic agent against mosquito larvae. The results were characterized based on the structural characteristics and physicochemical properties of the molecules.

Acknowledgements: Biobyte Corp., 201 West 4th St, Suite 204, Claremont, CA 91711, USA.

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P081

Novel Quinazoline and Pyrido[2,3-d]pyrimidine Hydroselenite Salts as Potent Antitumoral Compounds In Vitro Against Prostate Cancer

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Quinazoline derivatives have attracted attention due to their broad range of pharmacological activities, which include, among others, antifungal, antimalarial, anti-inflammatory and anticancer activity. These nuclei have emerged as versatile templates for a diverse range of mechanisms of anticancer activity and, considering our experience

with these heteroaromatic rings,^[1] we describe here the synthesis and biological evaluation of five novel quinazoline and pyrido[2,3-*d*]pyrimidine hydroselenite salts (Figure 1).

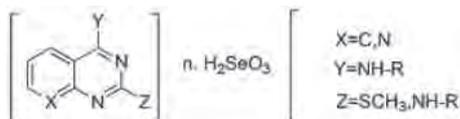


Figure 1. General structure of novel hydroselenite salt derivatives.

These hydroselenite salt derivatives were evaluated *in vitro* at 72 hours using the MTT assay against PC-3 (metastatic prostate cancer) cell line. All compounds presented IC₅₀ values below 10 μM (ranging from 1.67 to 7.00 μM). Owing to these promising cytotoxic values and in order to clarify the possible mechanism of action for these compounds, we investigated the ability of one pyrido[2,3-*d*]pyrimidine hydroselenite salt to activate caspase-3 (activation of caspase-3 is a key feature of apoptosis) and the effect to the cell cycle distribution at 24 and 48 hours. We reported a significant activation of caspase-3 and an increase in subG₀/G₁, S (very significantly), and G₂/M phases, with a significant reduction of the G₀/G₁ phase at 24 hours. Nevertheless, these effects disappeared at 48 hours.

The introduction of a hydroselenite group in quinazoline and pyrido[2,3-*d*]pyrimidine scaffolds maintained or increased their cytotoxic effects. Furthermore, the hydroselenite group seems to effect the mechanism of action. The selected hydroselenite derivative showed an activation of caspase-3 and a cell cycle effect at 24 hours, whilst its pyrido[2,3-*d*]pyrimidine analogue did not effect caspase-3 or the cell cycle.

We concluded that the formation of hydroselenite salts of quinazoline and pyrido[2,3-*d*]pyrimidine could be a valid approach to maintain or increase the activity and to modulate the mechanism of action for these scaffolds.

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P082

Homology Modeling of Human Voltage-Gated Sodium Channels and Binding Mode Studies of Modulators

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Voltage-gated sodium channels (VGSC) are complex membrane proteins that are widely expressed in neuronal, neuroendocrine, skeletal muscle and cardiac cells. They play a central role in the initiation and propagation of action potentials in electrically excitable cells. They activate in response to membrane depolarization and are responsible for the rapid influx of sodium ions during the rising phase of the action potential. The VGSCs are a family of heteromeric protein complexes consisting of four homologous domains (DI–DIV) of six transmembrane segments (S1–S6) as a pore-forming α-subunit in association with one or more β-subunits. The permeation pore is positioned at the extracellular side of the cell membrane and is formed by S5 and S6, linked by P-loops that fold partly back into the membrane to form the outer vestibule. The later hosts the selectivity filter (DEKA motif), which comprises four different amino acid side chains, one from each domain: Asp (DI), Glu (DII), Lys (DIII) and Ala (DIV).^[1]

To date, nine functional members of the family have been described (Na_v 1.1–1.9) with high degree of sequence homology, which translates into similar biophysical and pharmacological properties. Drugs targeting VGSCs are local anesthetics, antidysrhythmics and anticonvulsants, which provide good clinical efficacy driven through blockade of these channels; however, they show weak affinity and poor selectivity between channel subtypes. Consequently, this has led to search for subtype-selective modulators endowed with improved clinical efficacy and better toleration.^[2]

The first crystal structure of voltage-gated sodium channel from bacteria *Arcobacter butzleri* (Na_v Ab) in closed-pore conformation was recently solved^[3] and represents a good template for generation of homology models of different human VGSC (hNa_v 1.1–1.9). We have modeled the three-dimensional structures of hNa_v 1.3, hNa_v 1.4 and hNa_v 1.7 in closed and open conformations using closed Na_v Ab or open NaK^[4] channel structure as a template, respectively. The binding modes of tetrodotoxin, local anesthetics and other VGSC modulators were studied by docking into the generated homology models. Additionally, the models have been used in the design of analogues of clathrodin,^[5] an alkaloid toxin from tropical marine sponges.

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P083

Planning, Assay and In Silico Optimization of New Acetylcholinesterase Inhibitor's LeadsJonathan Resende de Almeida,^[a]Carmem Lúcia Cardoso,^[b]Carlos Henrique Tomich de Paula da Silva^[a]

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Alzheimer's disease (AD) is the most common type of dementia, and is responsible for 60–80% of cases. The early clinical symptoms include difficulty remembering names and recent events, as well as apathy and depression. Later symptoms are characterized by impaired judgment, disorientation, confusion, behavioral changes and difficulty in speaking, swallowing and walking.^[1] Also regarding the disease, several lines of evidence suggest that cholinergic deficits may contribute to the pathophysiology of AD. A deficit in cholinergic neurotransmission was established as a central feature in Alzheimer's disease pathophysiology. Thus, inhibition of acetylcholinesterase (AChE) is the most successful strategy for the current treatments for disease symptoms.^[2] The approved therapies for the treatment of AD are based on AChE inhibitors which maintain high levels of acetylcholine on muscarinic and nicotinic receptors in the central nervous system, which makes it an excellent target for drug development for use in the treatment of AD.^[3] The goal of this work is to design, test and optimize pharmacokinetic and pharmacodynamic properties of new compound prototypes as future drug candidates in AD. Through the search for AChE inhibitors in web server BindingDB (<http://www.bindingdb.org>), a considerable number of ligands to AChE was found. Other techniques such as the derivation of the pharmacophore, determining the molecular structural scaffold, presenting minimum molecular structural features and efficient interaction with the binding site, virtual screening simulations, and calculation of molecular interaction fields (mif) to map the interaction capabilities of the enzyme, were used for the design of new inhibitors of the enzyme, thus studying the pharmacokinetic profile of the identified compounds, trying to select those with a more appropriate profile, and optimize compounds with unfavorable properties. After selecting the compounds in a database by virtual screening and applying various filters for selecting the best inhibitors, some of these were obtained commercially, and enzymatic activity assays performed subsequently were made according to the Ellman's method previously described and modified.^[4] This method is based on measuring the rate of production of thiocholine formed by hydrolysis of the substrate analogue of AChE, the acetylthiocholine. The compounds showed a slight inhibition of the immobilized enzyme and, from these results; there will be an optimization of the leads.

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P084

Striving for Success in Antioxidant Therapy for Neurodegenerative Diseases: Current Status and Future Prospects

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The role and beneficial effects of natural antioxidants against various oxidative diseases have received great attention as they can exhibit potent antioxidant activity throughout different mechanisms such as scavenging ROS and RNS, binding to pro-oxidant transition metals (mainly Cu and Fe) and inhibiting ROS/RNS-generating enzymatic systems. In fact, the combination of these mechanisms can hinder the initiation and/or progression of free radical formation blocking or minimizing the oxidative damage cascade. In addition, their significance was supported by several epidemiological studies that have disclose an inverse relationship between dietary intake of phenolic antioxidants and the occurrence of diseases such as cancer and neurodegenerative diseases.

Until the date, the majority of natural antioxidants studied have attained limited therapeutic success a fact that could be related with their limited distribution throughout the body and with the inherent difficulties to attain the target sites. In fact, antioxidant therapies have enjoyed general success in preclinical studies across animal models, but little benefit in human intervention studies or clinical trials. Actually, at the molecular level, a synchronized system of transporters, channels, receptors and enzymes act as gatekeepers to foreign molecules. So, an effort to eradicate or improve antioxidants with problematic ADME/Tox profiles must be performed and, if the conditions are met natural modified compounds can efficiently operate as potent exogenous antioxidants and in that way supplement the body's endogenous antioxidant defense systems. The results so far obtained confirm the importance of exploring natural phenolic systems as safer templates to build through rational design approaches new antioxidant candidates, namely

mitochondriotropic compounds based in natural antioxidants present in diet. These new antioxidants could be used as potent and selective agents throughout specific targeting the mitochondria in neurodegenerative diseases therapy. The driving efforts performed so far to improve the nature's design strategy will be outlined in the present communication.

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P085

Computational Studies of Human Cathepsin L Inhibition

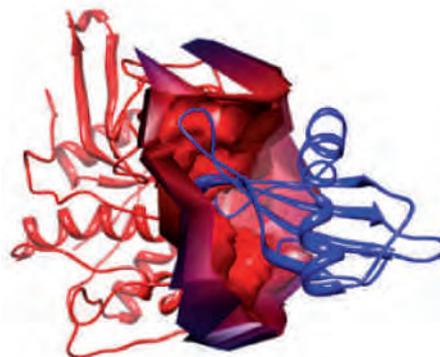
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Cathepsin L is widely distributed in the cell; particularly in the lysosome, in the nucleus, and in the extracellular compartment. This enzyme is responsible for different processes, depending on its particular location. During the last decade a strong evidence for the participation in cancer diffusion by cathepsins (in particular cysteine proteases) has emerged. Proteases secreted by cancer cells have been proposed to facilitate tumor invasion and metastasis by degrading the basal membranes components such as collagen, elastin, cadherins and other structural proteins.^[1,2] Recent studies showed that cathepsin L, in particular, is involved in these last processes. Indeed cathepsin L activity increases during tumorigenesis while its inhibition decreases tumor development. Therefore, cathepsin L was recently suggested to be an attractive target for the development of anticancer-antimetastatic agents.

Moreover, in some cancers, the changes in cathepsin L expression or activity has been shown to have high diagnostic power.^[3,4] It is also known that stefin B is an endogenous inhibitor of cathepsin L. Based on that, investigating the interactions between stefin B and cathepsin L in silico is expected to supply a valuable knowledge enabling us to improve both diagnosis and therapy. In addition, several isoquinoline alkaloids, which are comprised in a prepara-

tion (Ukraine) patented in several countries but never approved by the FDA (reported to possess interesting anticancer-antimetastatic properties), appear to be worth of analogous studies. A computational approach was used to obtain a three-dimensional (3D) model of a cathepsin L–stefin B complex in silico, which was exploited to investigate at a molecular level the interaction between the two partners through a molecular docking method.



Moreover, in view of estimating in silico potential inhibitory properties of the alkaloids mentioned above, their interactions with the theoretical model of cathepsin L obtained were also investigated through the same molecular docking protocol.

The structure analysis of the best docking poses gave in both cases interesting suggestions, which can be exploited for the design of new effective inhibitors of cathepsin L, to be proposed as anticancer–antimetastatic drugs.

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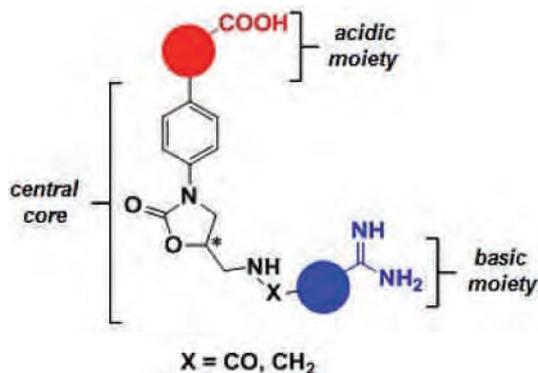
P086

Design, Synthesis and Biological Evaluation of Novel Dual Antithrombotic Compounds – Inhibitors of Factor Xa and Antagonists of GPIIb/IIIa

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Cardiovascular diseases, such as myocardial infarction, stroke, unstable angina pectoris and pulmonary embolism, are a major cause of mortality in the developed world.^[1] As a consequence of proven synergistic effects observed during therapies exploiting combinations of anticoagulants and antiplatelet drugs,^[2,3] dual antithrombotic agents targeting combinations of different coagulation enzymes and platelet receptors have started to emerge. Based on previous work in our group that led to promising agents combining thrombin inhibitory and glycoprotein IIb/IIIa receptor antagonistic activity,^[4] we decided to attempt the design of novel dual antithrombotic agents combining factor Xa inhibitory and glycoprotein IIb/IIIa receptor antagonistic activity. Known crystal structures of factor Xa in complex with rivaroxaban, its potent direct inhibitor^[5,6] and GP IIb/IIIa cocrystallized with its antagonist tirofiban^[7,8] were used for docking of virtually designed molecules combining pharmacophores of rivaroxaban and RGD sequence possessing an anionic and a basic center in appropriate distance, which is responsible for recognition and binding of various adhesive endogenous protein ligands to GP IIb/IIIa. According to the results of docking and accessible synthetic options, we prepared novel molecules consisting of various moieties bearing a carboxylic acid group as an anionic center and moieties bearing a basic center attached to opposite sides of the rivaroxaban central core (shown). Biological evaluation of the given compounds including determination of K_i values on factor Xa^[9] and IC_{50} values on GP IIb/IIIa,^[10] as well as a study of selectivity on serine proteases by determination of the K_i values on thrombin^[9] and trypsin^[9] gained insight into structure–activity relationships, which will be used in further optimisations towards novel dual antithrombotic compounds.



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P087

Anticonvulsant Activity of Ether Derivatives of (Homo)piperidines – Histamine H₃ Receptor Antagonists

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Epilepsy is a brain disorder characterized by repeated seizures over time. Convulsions are episodes of disturbed brain activity that cause changes in attention or behavior. It is a public health problem that affects approximately 1% of the world population. The seizures are associated with neuronal hyperactivity and showed imbalance between excitatory glutaminergic signaling and inhibitory GABAergic signaling. Antiepileptic drugs (AEDs) can influence the inhibitory or excitatory neurotransmitter systems (GABA or glutamic and aspartic acid, respectively), or the ion transport across cell membranes.

In the nineties, it was demonstrated that the central histaminergic neuronal system plays an important role in the inhibition of seizure activity. Some studies reported that histamine H₃ receptor (H₃R) inhibition reduces epileptic symptoms in various animal models, e.g., in the maximal electroshock (MES), kindling and subcutaneous pentylenetetrazole-induced convulsions (ScMet).^[1,2] It is expected that H₃R antagonists, alone or in combination with AEDs, could contribute to the treatment of epilepsy, especially in patients with inadequate response to the conventional therapy.

With the aim to continue our previous works in the H₃R field,^[3,4] we prepared a series of (homo)piperidine and piperazine ether derivatives, which were tested for human H₃R affinity and were evaluated by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Health in Bethesda, USA. H₃R affinities were evaluated in binding assay at the human hH₃R expressed in CHO-K1 or HEK 293 cells stably transfected with the full-length coding sequence of the hH₃R.^[5,6] Anticonvulsant properties of the obtained compounds were evaluated in two major convulsant tests: MES and ScMet. In addition, neurological toxicity was evaluated in mice using rotarod test.^[7]

The obtained compounds showed moderate to high affinity at hH₃R (K_i values from 326 to 9 nM). The majority of them were active in the MES test at a 100 mg/kg dose 15 or 30 min after i.p. administration to mice and were inactive in the ScMet test. All compounds showed some signs of neurotoxicity in high doses.

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P088

The Capillary Isoelectric Focusing: A Novel Approach for the Fast Determination of pK_a Values of Small Compounds

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Inappropriate ADME (absorption, distribution, metabolism and excretion) behaviour leads to the rejection of new chemical entities (NCEs) during drug development. Thus, the determination of physicochemical properties such as lipophilicity or solubility is of crucial importance in the early steps of drug discovery. Ionization constants are also widely determined since the ionization states govern other physicochemical and pharmacokinetic properties. Nowadays, pK_a values are determined by powerful methods such as potentiometry, spectrophotometry or capillary zone electrophoresis.^[1]

Capillary isoelectric focusing (cIEF) is an electrophoretic technique allowing to separate polyelectrolytes according to their isoelectric point (pI), i.e., the pH at which an amphoteric compound is under its neutral form, using a pH gradient created within a capillary. cIEF is

employed to separate proteins in proteomic applications, analysis of complex protein mixtures and microheterogeneity determination.^[2] The aim of this study was to evaluate the potential of cIEF for the determination of pK_a of small compounds in one injection from the determination of the pH range where compounds are under their neutral form.

Simple and monofunctional compounds were used to explore the performance of this approach. The time corresponding to the apparition of the neutral form was collected and compared to the pK_a value from the literature. Linear correlations were obtained ($r^2=0.997$, slope=-3.67, intercept=55.00 and $r^2=0.998$, slope=-2.80, intercept=43.94 for five acidic and five basic compounds, respectively). These relations can be used as calibration curves for the pK_a determination of unknown compounds. In conclusion, capillary isoelectric focusing was successfully used for the rapid determination of pK_a values of simple and monofunctional compounds and suggests interesting perspectives for early drug discovery.

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P089

Novel FRET-Based Approaches to GPCR Drug Screening and Functional Architecture Studies

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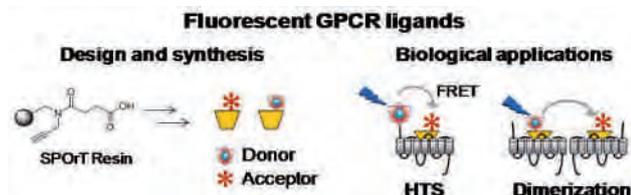
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G protein-coupled receptors (GPCR) represent the most important class of therapeutic targets in the pharmaceutical industry. It is of importance to gain a better understanding of their functioning, their molecular structure but also to set up new receptor-selective high-throughput screening (HTS) assays. Owing to their high sensitivity and to their reduced environmental safety risk, fluorescent technologies represent a powerful molecular tool to perform these studies. Among these techniques, fluorescence resonance energy transfer (FRET) between a fluorescent donor–acceptor pair has been shown to be a convenient method to investigate intra- and intermolecular interaction processes both in vitro and in vivo.^[1]

In this context, we have developed synthetic methods to facilitate the access to fluorescent GPCR probes^[2] both to accelerate GPCR drug screening and to gain a better understanding of their functional architecture. Applications will be presented: 1) the preparation of a fluorescent compound-based library, its screening on EGFP-fused apelin GPCR and the identification of the first nonpeptidic agonist of this receptor;^[3] 2) the design and synthesis of the first selective fluorescent nonpeptidic vasopressin V₂ receptor antagonists for GPCR oligomerization studies at the surface of living cells.^[4]



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P090

Novel 6,7-Methylenedioxy-4-amino-quinazolinic Analogues Designed as EGFR Inhibitors

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The protein kinases that phosphorylate tyrosine (Tyr) residues in target proteins are known as tyrosine kinases. These proteins are subdivided into cytoplasmic non-receptor tyrosine kinases, which are regulated by several mechanisms; and transmembrane receptor tyrosine kinases, which are activated by an extracellular ligand. The kinase domain, conserved throughout the class, is responsible for the catalytic activity.^[1] Some tyrosine kinases are known to be activated and/or overexpressed in tumor cells, promoting tumor growth and progression. Thus, inhibiting the kinase activity of these proteins is considered a promising therapeutic strategy for cancer therapy.^[2,3]

The epidermal growth factor receptor (EGFR or ErbB1) belongs to the family of tyrosine kinase receptors of growth factors. Overexpression of members of this family, e.g., ErbB1 and ErbB2, is observed in

several types of solid tumors, and it is associated with an unfavorable prognosis.^[1,2] Some 4-amino-quinazolinic EGFR inhibitors are available in the pharmaceutical market, i.e., gefitinib, lapatinib and erlotinib.^[4] However, besides the limited therapeutic alternatives and the high cost of these medicines, reports of resistance development to these drugs have been described in the literature.^[5] Thus, there still exists a great interest from researchers on the identification of new EGFR inhibitors useful in cancer treatment.

In recent years, the 4-amino-quinazoline nucleus has been highlighted as a versatile structural scaffold in the design of new bioactive compounds through the modulation of a wide range of biological targets. This privileged structural pattern can be appropriately directed to different target proteins through suitable addition of substituents.^[2,6]

The design concept of the novel 6,7-methylenedioxy-4-amino-quinazolinic analogues was based on the molecular hybridization between the privileged structure of 4-amino-quinazolinic nucleus and the benzodioxole core, also described as an useful scaffold for the design of new compounds directed to several therapeutic targets.^[7] The resulting 6,7-methylenedioxy-4-amino-quinazoline molecular pattern was then functionalized in positions 2 and 4 of the quinazolinic ring, guiding the affinity to the selected therapeutic target, i.e., EGFR.

The designed compounds were synthesized through a key step Buchwald-Hartwig reaction^[8] for the insertion of the amino group in position 4 of the 6,7-methylenedioxy-quinazoline scaffold via the palladium-catalyzed cross-coupling of functionalized anilines with the aryl halide key intermediate.

The synthesized compounds were tested in a panel of kinases considered relevant for cancer treatment, i.e., EGFR, VEGFR2, EGFR L585R and B-Raf V600E.

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P091

Alkylimidazo-, Pyrimido- and Diazepinopurinediones as Adenosine Receptor Ligands

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Adenosine modulates a variety of important physiological processes and exhibits central nervous system depressant, cardiodepressant, antidiuretic and immunomodulatory effects. To date, four adenosine receptor (AR) subtypes, A₁, A_{2A}, A_{2B} and A₃, have been cloned and pharmacologically characterized. These receptors belong to the superfamily of G protein-coupled receptors. A₁ adenosine receptor antagonists are investigated as cognition enhancers, for therapeutic use in dementias, such as Alzheimer's disease, as antihypertensives and potassium-saving diuretics with kidney protective effects, for the treatment of depression, asthma and the prevention of ischemia-induced injuries. Numerous studies have confirmed the ability of A_{2A} adenosine receptor antagonists to improve the symptoms of Parkinson's disease in animal models and even to prevent neurodegeneration and ischemic brain damage. They may also be beneficial for the treatment of epilepsy.

Our efforts were directed towards the development of new selective adenosine receptor antagonists with a tricyclic xanthine structure. The most active A₁ AR ligands were found among the 1,3-dipropyl-substituted benzylpyrimidopurinediones, while A_{2A} adenosine receptor ligands were typically 1,3-dimethyl-substituted aryl-, cycloalkyl- and phenalkyl-pyrimidopurinediones.^[1] Several of the most active ligands at adenosine A_{2A} AR demonstrated antiparkinsonian effects.^[2] As a continuation of our search for potent adenosine A₁ and A_{2A} receptor ligands, we have developed a new series of imidazo-, pyrimido- and diazepino[2,1-f]purinedione derivatives with aliphatic substituents in the annelated ring, e.g., alkyl, alkynyl and alkenyl chains. The obtained derivatives (1–29) were evaluated for their affinity at A₁ AR at rat brain cortical membranes and at A_{2A} AR at rat brain striatal membranes. Additionally their affinity at human recombinant A₁, A_{2A}, A_{2B} and A₃ adenosine receptors was examined. Evaluated compounds have shown affinity towards A₁ AR and/or A_{2A} AR. The most potent ones were those with butyl substituents attached to pyrimido- and diazepinopurinedione core structure. X-ray structure analysis was performed for three derivatives. The obtained results were used for molecular modeling studies, and receptor docking studies were performed.

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P092

Fighting Cancer with Visible Light: New Applications for Metal Complexes in Photoactivated Cancer Therapy

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Light as a therapeutic instrument is common since the ancient Egyptians and it is still a promising approach to attain the site-specific activation of chemotherapeutics in modern cancer therapy. Especially porphyrins found their way into clinical photodynamic therapy and are now successfully used as drugs in skin,^[1] lung,^[2] bladder^[3] and esophageal^[4] cancers. The great advantage of light activated cancer therapy is spatial and temporal control of the toxic effect induced by the chemotherapeutic agent which minimizes the enormous side effects of common chemotherapy. Because of their unique photochemical and photophysical properties transition-metal complexes offer new prospects for this kind of cancer treatment. The availability of a large variety of easily accessible electronic excited states which may be used for the photoinduction of ligand dissociations, substitutions, rearrangements, redox reactions, or even catalytic processes is an important characteristic which discriminates transition-metal complexes from pure organic compounds.^[5]

With the aim to find metal complexes with new biological properties our group found a new class of organometallic compounds with amazing light activated cytotoxicity in human cancer cell lines. After irradiation with visible light we can see an increase in the cytotoxicity of the compound by a factor of 1000. Further studies reveal that a concentration of 1 μM and an irradiation time of 20 min are sufficient to induce apoptosis in about 90% of HeLa cells as well as in multiresistant colon carcinoma cells (HT29). While first results indicate that the cytotoxic effect is different from that of classic photodynamic therapy, this complexes could be promising candidates to open new ways in photoactivated cancer therapy.

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P093

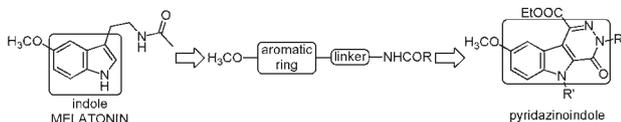
Novel Pyridazinoindole Derivatives as Melatonin Receptor Agonists

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Melatonin (MLT) is a neurohormone produced in mammals by the pineal gland mainly during darkness and characterized by a circadian rhythm of secretion. Its chronobiotic and sleep-inducing properties have led to MLT as the principal neurochemical agent involved in insomnia and circadian-rhythm-related disorders.^[1] MLT acts through two main GPCR receptors, MT₁ and MT₂, which have become two of the most promising pharmacological targets for sleep regulation. Due to limited use of melatonin as a drug by its short half-life and its poor availability, a great interest has been drawn to the discovery of new agonists of MLT receptors. Although many research groups have focused their efforts on obtaining melatonin receptor ligands,^[2] only two melatonin agonists are on the market: ramelteon and agomelatine.

A methoxy group and *N*-alkylamide chain attached to the central aromatic scaffold by an aliphatic linker have been shown to be important for binding to the receptor. As part of our research of melatonin agonists and based on our expertise on pyridazinoindoles chemistry,^[3] we designed and synthesized a novel class of potential melatonin analogues.



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P094

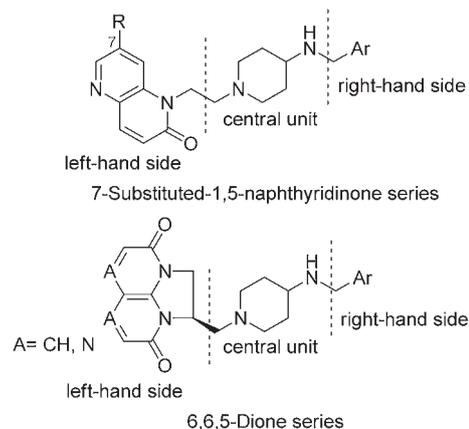
Mycobacterium tuberculosis DNA Gyrase Inhibitors: Building Oral Developable Antituberculars

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Tuberculosis has become one of the most extended diseases around the world. Shortening of the current treatment as well as new drugs effective against increasingly appearing resistant strains are urgently needed. A new family of DNA Gyrase inhibitors with a different mode of action to Fluoroquinolones, and therefore not cross-resistant, has been developed at GSK. Herein, we present our progress in shaping these compounds in terms of compound quality (oral drug-likeness profile) and anti-TB potency, towards candidate selection.

Screening against *Mycobacterium tuberculosis* (Mtb) of a subset of compounds selected from the GSK gyrase inhibitors collection enabled us to identify 7-substituted-1,5-naphthyridones as a starting point. Variation of the substituents in position 7 had a significant impact on the activity and metabolic stability of the compounds. Incorporation of monocyclic aromatic moieties in the right-hand side of the molecule proved to be optimal for a selective anti-Mtb profile.



A potential cardiotoxicity liability related to hERG inhibition was initially encountered and a correlation with lipophilicity was observed. Optimization led us to a new series of more polar compounds having a 6,6,5-dione in the left-hand side. This family possesses a more balanced profile in terms of activity, metabolism and safety.

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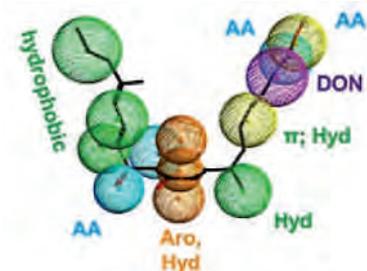
P095

Is RNAP a Suitable Target for CADD? RNAP–Myxopyronin Complexes as Starting Points for MD Simulations, Homology Modeling and 3D-Pharmacophore Virtual Screening

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Bacterial RNA polymerase (RNAP) is a large complex consisting of four different subunits forming the core enzyme ($\alpha_2\beta\beta'\omega$), which is dependent on a sigma factor (σ) for promoter recognition and transcription initiation (holoenzyme). The structural diversity between bacteria and eukaryotes makes RNAP an interesting target for the development of broad-spectrum antibiotics. Several binding sites for RNAP inhibitors have been reported. One of them is the “switch” region, which mediates opening and closing of the active center cleft and which is occupied by myxopyronins, potent inhibitors of RNAP.



Myxopyronins-derived features shown (structure-based features hidden)

In this work, we focused on the “switch” region of RNAP and on its role as binding site for potent RNAP inhibitors. Two *T. thermophilus* RNAP–inhibitor complexes ($\alpha_2\beta\beta'\sigma$; PDB ID: 3dxj with myxopyronin and 3eqj with 8-desmethyl-myxopyronin) were investigated in a comparative MD simulation approach. Binding free energies for the inhibitors were predicted and compared to their IC_{50} values. In a “macro-to-micro” perspective, we also performed a set of MD simulations for one of the RNAP–inhibitor complexes with reduced subunit complexity of the RNAP ($\beta\beta'$, $\beta'\sigma$, $\beta\beta'\sigma$). In parallel, a homology model of *E. coli* RNAP was built. Taking into account the inhibitors, as well as the structural variability seen in the MD simulations and the homology models, we generated a 3D-pharmacophore model with MOE, used for virtual screening. 70 virtual hits were selected and tested for their in vitro RNAP inhibitory potency. Three hits were identified and used as starting points for optimization, which finally resulted in novel RNAP inhibitors with IC_{50} values around 10 μ M.

P096

Pharmacophore Development for the Discovery of New Alpha-Amylase Inhibitors

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Alpha-amylase enzyme plays role in catalyzing the hydrolysis of alpha-(1,4)-glycosidic linkage in starch leading to increasing the postprandial blood glucose levels.^[1] So, it is one of the best targets for development of therapeutic agents for diabetes II and obesity. It is known that most of alpha-amylase inhibitors are carbohydrates and derivatives with undesirable properties for oral application. So, the goal of this study was the developing of a tool for identifying new alpha-amylase inhibitors with drug-like properties.

To attain our goal, different structural features of the co-crystallized ligands with the enzyme available in PDB^[2] were studied and the essential chemical features for inhibition were analyzed using LigandScout.^[3] Study showed that subsites in the active site cleft labeled as -1,+1 constitute the cornerstone for alpha-amylase binding where the catalytic triad (D197, E233, D300) is present. Hence, different pharmacophores were developed and validated against databases composed of known biologically 19-active and 55-inactive alpha-amylase inhibitors, which were collected from literature and the ChEMBL database.^[4]

A final 3D pharmacophore model with essential features required for enzyme inhibition was obtained. Using the Receiver Operating Characteristic plot,^[5] the obtained model showed true positive rate of 63% with 1.8% false-positive rate along with an AUC value of 0.81. Screening different commercial chemical databases with this model showed interesting non-carbohydrate scaffolds that fit the obtained model. The newly promising structures will be further examined using structure-based design and biologically evaluated.

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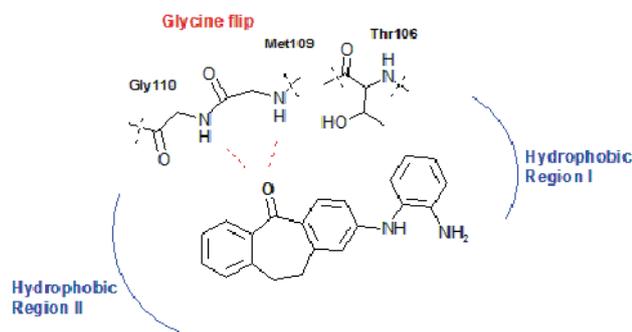
P097

Design, Synthesis and SAR of Phenylamino-Substituted 5,11-Dihydrodibenzo[*a,d*]-cyclohepten-10-ones and 11*H*-Dibenzo[*b,f*]-oxepin-10-ones as p38 MAP Kinase Inhibitors

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The p38 MAP kinase is a key player in signalling pathways regulating the biosynthesis of pro-inflammatory cytokines. Small-molecule p38 inhibitors suppress the production of these cytokines making p38 a promising drug target for novel anti-inflammatory drugs.



We recently reported a novel series of dibenzepinone inhibitors belonging to the class of so called linear binders. Upon binding of the dibenzepinone inhibitor, the p38 MAP kinase undergoes a Gly110 flip. The glycine flip is a small conformational rearrangement in the hinge region of the p38 MAP kinase induced by the inhibitor, and it provides selectivity for p38 α over other kinases with less flexible, non-glycine residues at this position. Hence the carbonyl functionality of the dibenzepinone inhibitor is essential for any inhibitory activity and selectivity as it forms two hydrogen bonds towards Met109 and Gly110 in the hinge region.

In this study, we report the design, synthesis, and SAR of novel *N*-substituted 11*H*-dibenzo[*b,f*]oxepin-10-ones and 5,11-dihydrodibenzo[*a,d*]cyclohepten-10-ones as p38 inhibitors. Our aim was to retain the key interaction: the bidentate hydrogen bond of the carbonyl oxygen of the inhibitor to the backbone NH of Met109 and the backbone NH of Gly110. Docking studies predicted alternative positions for the carbonyl oxygen of the inhibitor. Within these inhibitor structures, the carbonyl functionality was moved on the bridge side and the linker atoms X and Y were varied to obtain distinctive molecular geometries and to obtain additional interaction opportunities.

Initial investigations of the inhibitory activities and structure-activity relationships of phenylamino-substituted dibenzo[*b,f*]oxepin-10(11*H*)-one, 5,11-dihydro-(10*H*)-dibenzo[*a,d*]cyclohepten-10-one and dibenzo[*b,f*][1,4]oxazepin-11(10*H*)-one inhibitors for p38 MAP kinase were accomplished. The promising structural variations suggested by our docking experiments did not result in any novel compounds as active as the lead compound. While our initial hypothesis

that substitution of a tricyclic scaffold would result in a favorable position for the carbonyl functionality in the inhibitor was not supported by our data, we did identify some structural determinants that may be useful for the development of future p38 MAPK inhibitors.

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P098

Synthetic Mimicry of Protein Binding Sites through Structure-Based Design and Computational Optimization

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Molecules capable of mimicking protein binding and/or functional sites present useful tools for a range of biomedical applications, including the inhibition of protein–ligand interactions. Mimetics of large and sequentially discontinuous protein binding sites can presently be generated through structure-based design and chemical synthesis.^[1] The goal of computational protein design, on the other hand, is to improve protein binding affinity and/or specificity by predicting appropriate mutations at protein–protein interfaces.^[2]

The aim of this project was to explore the potential synergism resulting from combining these two strategies by (i) designing and generating synthetic mimetics of a conformationally defined protein binding site and (ii) optimizing these molecules, regarding their affinities to the protein ligand, through computational design. The well-known interaction of the synaptic enzyme acetylcholinesterase (AChE) with its inhibitor fasciculin-2 (FAS) served as a model for this study.

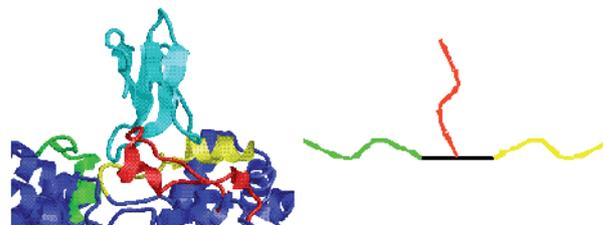


Figure 1. Left) Crystal structure of hAChE (blue, discontinuous binding site marked in green, red and yellow) complexed with FAS II (cyan).^[3] Right) Assembled peptide presenting the hAChE binding site fragments.

Assembled peptides mimicking the discontinuous binding site of hAChE for FAS, which were designed based on the crystal structure of a hAChE–FAS complex (Figure 1),^[3] were found to specifically interact with FAS. The affinity to FAS could be enhanced by introducing single-point mutations, which were proposed through computational design.

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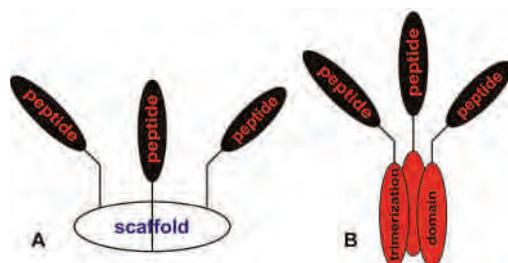
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Trivalent Presentation of Synthetic HIV-1 GP120-Derived Peptides

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Peptides presenting the binding sites of viral surface proteins for their cellular receptors are promising candidates as entry inhibitors, as well as immunogens to elicit a virus neutralizing immune response. The HIV-1 glycoproteins gp120 and gp41 form C₃ symmetrical trimeric spikes on the virus surface.^[1] Therefore, trivalent presentation of gp120-derived peptides may enhance their avidity and affinity in the interaction with the receptors (CD4 and coreceptors, respectively).



For the generation of C₃ symmetrical trimeric peptides, we either use functionalized scaffolds, to which the peptides are covalently attached (Figure 1A), or couple the peptide to trimerization domains of proteins, such as the foldon domain of bacteriophage T4 fibrin^[2] or the gp41 ectodomain,^[3] which fold into very stable, non-covalent trimers (Figure 1B). Using these methods, and based on a 3D model of trimeric HIV-1 spikes,^[1] peptides presenting the CD4 binding site^[4] or the V3 loop, respectively, of gp120 were generated as non-covalent and covalent trimers. The affinities of these trimeric peptide conjugates to CD4 and antibodies recognizing the CD4 binding site or the V3 loop of gp120 were then compared to the respective monomers.

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P100

Study of the E₀ Region of Anaplastic Lymphoma Kinase

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that belongs to the superfamily of insulin receptors. During the past decades ALK aroused interest as target for anticancer therapy.^[1] Originally identified as a chromosomal translocation between ALK and the nucleophosmin (NPM) gene, the resulting NPM-ALK fusion oncogene product leads to disorganisation of differentiation, cell cycle perturbation and apoptosis.^[2]

Kinoshita et al. recently reported benzo[d]carbazole derivatives as ALK inhibitors with low nanomolar IC₅₀ values.^[3] The methodologies applied by the authors for kinase selectivity optimization are based on exploitation of the E₀ region. Since ALK owns relatively small amino acids (alanine and glycine) within this region, selectivity can be gained by introducing appropriate substituents into the inhibitor molecules. We used in silico tools including the protein–ligand docking programs GOLD and MOE to demonstrate the feasibility of this approach. Firstly, we were able to explain differences of inhibitor potencies as a matter of structural properties of the tested compounds. Subsequently, the selectivity of the test compounds against ALK versus an exemplary kinase (VEGFR-2) could be rationalized by the applied methods. The results underscore the relevance of the E₀ region for the further design of novel selective ALK inhibitors.

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P101

New Inhibitors for Nucleotide-Binding Proteins

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The synthesis of selective inhibitors represents a fundamental field of medicinal chemistry. In contrast to many other groups who prepare purely organic compounds with biological activity, Meggers, et al. have been focusing on organometallic compounds as enzyme inhibitors. These compounds comprise a pharmacophore ligand, a metal center and various ligands to fulfill the remaining coordination sites. The resulting inert and rigid metal complexes show some interesting features: The pharmacophore ligand plays a major role for the inhibitor recognition, and the metal center allows the design of a sophisticated architecture through its ability to act as an octahedral center overcoming the limitations of the usual tetrahedral geometry of purely organic molecules. The vast number of potential ligands around the metal center gives rise to a highly diverse library of compounds which can be tailored in a rather easy fashion. Up to now, this concept has been proven successful for protein kinases, and hence a series of highly potent and selective inhibitors were published by our group.^[1]

One part of our on-going research focuses on the design and synthesis of new pharmacophore chelate ligands. Inspired by the structural features of adenosine, such as its H-bond-donor/acceptor pattern incorporated in a flat and extended aromatic system, new scaffolds can be envisioned. In this approach, the bulky structure of the ribose unit is replaced by the metal center. The resulting inhibitors might not be limited to kinases, but could broaden the application to the enormous, yet neglected, enzyme family of ATPases. Being involved in many cellular processes at the origin of human diseases, they are generally interesting drug targets, and there is already a selection of ATPase inhibitors on the market. As ATP-competitive inhibition is challenging, most of the known inhibitors do not bind directly to the ATP-binding site in comparison with the approaches in kinase inhibition, although structural differences within the ATP-binding sites should allow the development of selective inhibitors in principle.^[2] Additionally, not only ATP-binding proteins but also other nucleotide-binding proteins could be addressed as drug targets this way.

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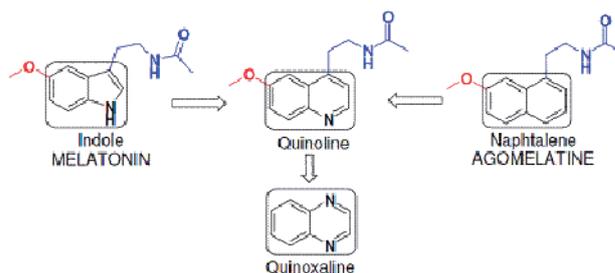
Design, Synthesis and Biological Evaluation of New Quinoxaline Derivatives as Melatonin Receptor Ligands

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Numerous studies on the role of MLT in modulation of the sleep-wake cycle and circadian rhythms in humans have been performed since the discovery of the circadian nature of melatonin (MLT) secretion. The two main MLT receptors involved in these functions, MT1 and MT2, were cloned a long time ago and well characterized. So, MLT receptor agonists are now appearing as new promising treatment options for sleep and circadian-rhythm-related disorders. Furthermore, four therapeutic agents (ramelteon, tasimelteon, prolonged-release MLT and agomelatine) are already in use.^[1]

The pharmacophore structure found in almost all MLT receptor agonists includes an amide group connected by a linker chain, to an aromatic nucleus carrying a methoxy group. Several potent MLT agonists have been designed by replacement of the indole core with other aromatic rings, such as the naphthalene system and the quinoxaline ring.^[2]



According to this structural approach, we introduced a quinoxaline ring, a bioisoster of naphthalene and quinoline, as the central core. In this work, we report the design, synthesis and biological evaluation of new potential MLT analogues.

Acknowledgements: We wish to express our gratitude to the Government of Navarre for the grant given to S. Ancizu.

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P103

Lead Optimization of Various Amido-Quaternary Ammonium Salts in Piperazine Alkyl Derivatives by Study of the Molecular Properties and In Vivo for Anticancer Therapy through RhoB-Mediating Pathway

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The protein kinase B (Akt) pathway is generally activated in cancer cells and has a wide range of downstream targets that regulate tumor-associated cell processes. Phosphatidylinositol (PI) analogues are one class of Akt inhibitors that is represented by perifosine and phosphatidylinositol ether lipid analogues (PIAs). Through the PI3K/Akt pathway, oncogenic Ras downregulates RhoB, which is a suppressor of transformation, invasion and metastasis of the cell. This prompted us to suggest that piperazine alkyl derivatives can induce apoptosis through the PI3K/Akt pathway, the RhoB mediated pathway, or both pathways. We synthesized novel series of RhoB modulators and evaluated their biological activities. The 568 synthesized analogues were assayed for antiproliferative activity against six different human cancer cell lines. Among these analogues, 118 active compounds were chosen with selectivity in prostate and gastric cancer cells. Although analogues related to the lead compound G02(NSC126188) showed good cancer-cell-growth inhibition under 0.5 µg/mL, poor in vivo tumor regression activities were observed due to the low plasma exposure. We select the compounds through molecular properties. Perifosine was used as reference of molecular properties because it already showed a high oral bioavailability and a long terminal half-life, and low toxicity (below 50 mg). Using five parameters, 32 compounds satisfied the criteria. Through oral administration of xenograft regression model, A895 emerged as the most promising anticancer compound by promoting apoptosis through the RhoB-mediated pathway, the PI3K/Akt pathway, or both.

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Acid-Modified NSAIDs from COX to mPGES-1 Inhibitors

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are still a common treatment for many inflammatory diseases but suffer from severe GI side effects. After the failure of COX-2 inhibitors great attention was focused on mPGES-1 inhibitors (3rd generation NSAIDs). mPGES-1 is

a major source of PGE₂ in inflammation, and its role in a number of diseases is well established. First leads for mPGES-1 inhibitors were derived from 5-LOX inhibitors such as MK886 (FLAP IC₅₀=26 nM). By modifying the carboxylic group of NSIADs by coupling them with different aryl and alkyl sulfonamides, we were able to reduce COX1/2 activity, but increase mPGES-1. 2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-tosylacetamide showed an IC₅₀ value for mPGES-1 of 6.4 µM and showed complete loss of COX activity. Further optimization of NSAIDs led to sub-micromolar mPGES-1 inhibition.

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P105

New Synthesis of Diazepino[3,2,1-*ij*]quinolone and Pyrido[1,2,3-*de*]quinoxalines via Addition–Elimination Followed by Cycloacylation: Possible Ligands for Cannabinoid Receptors

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In order to identify new cannabinoid ligands, we designed a hybrid chemical structure that includes the structural features of known cannabinoid ligands.^[1] This project describes a convenient and efficient synthesis of new fused tricyclic diazepino[3,2,1-*ij*]quinolines, and substituted pyrido[1,2,3-*de*]quinoxalines.^[2] *o*-Phenylenediamines are transformed in the tricycles nucleus in only a few-step synthetic sequence to produce ethyl 2,8-dioxo-1,2,3,4-tetrahydro-8H-[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxylate, ethyl 8-oxo-1,2,3,4-tetrahydro-8H-[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxylate and ethyl 2,7-dioxo-2,3-dihydro-1H,7H-pyrido[1,2,3-*de*]quinoxaline-6-carboxylate. The biological evaluation of synthesized compounds is in progress.

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Structure-Based Design of Highly Potent and Selective Cyclic Plasmin Inhibitors

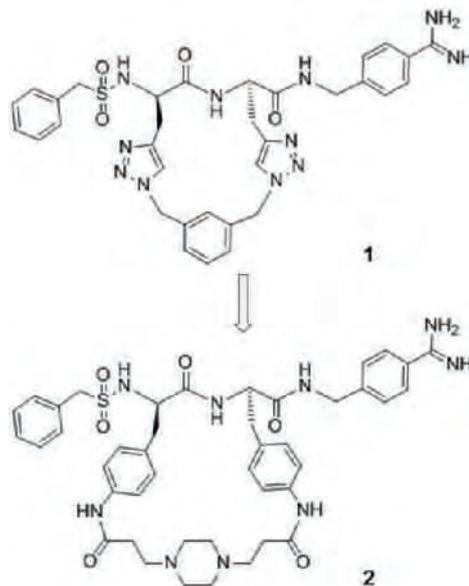
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The trypsin-like serine protease plasmin is responsible for the degradation of fibrin clots in blood. Therefore, plasmin inhibitors can be used for the treatment of hyperfibrinolysis, which may occur during cardiac surgery with cardiopulmonary bypass or organ transplantation. For many years, the 58 amino acids long peptidic plasmin inhibitor aprotinin was clinically used to reduce blood loss under these conditions. Due to later reported side effects, it was withdrawn from the market in 2008. Presently, only tranexamic acid or *p*-aminomethylbenzoic acid can be used as alternative antifibrinolytics. However, both compounds inhibit only the plasminogen activation, but have no direct inhibitory effect on already formed plasmin. Therefore, the development of new injectable plasmin inhibitors as replacement for aprotinin for use in cardiac surgery is of therapeutic interest.

In contrast to all over trypsin-like serine proteases, plasmin is missing a special loop segment around amino acid 99 in its active site. The direct connection between the plasmin residue 94 and the amino acid in position 101 is called 94-shunt and is a unique structural feature of plasmin. We have recently developed a first series of highly potent substrate-analogue plasmin inhibitors,^[1] which are cyclized between the side chains of their P3 and P2 amino acids. For example, compound **1** inhibits plasmin and plasma kallikrein with inhibition constants of 0.8 and 2.4 nM, whereas it has negligible activity against the related proteases thrombin, factor Xa, protein Ca, uPA or tPA, most likely due to sterical repulsion from the 99-loop present in the other trypsin-like serine proteases. However, compound **1** has relatively poor solubility, which might be a disadvantage for an injectable drug, and its synthesis requires the use of the potentially hazardous 1,3-bis(azidomethyl)benzen as intermediate.

Replacement of both triazoles in **1** by phenyl rings in combination with a piperazine-linker segment provided various inhibitors with improved affinity, selectivity, and high solubility. For example, compound **2** inhibits plasmin with a K_i value of 200 pM, has strong antifibrinolytic activity in plasma and no influence on blood coagulation. It possesses high metabolic stability when incubated with liver microsomes and has negligible affinity to various ion channels. Based on its excellent overall profile, inhibitor **2** could be a suitable candidate for further antifibrinolytic drug development.



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P107

Computer-Aided Discovery of Ligands for HIV-1 Frameshift-Inducing RNA Stem-Loop

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Human immunodeficiency virus type 1 (HIV-1) utilizes programmed -1 ribosomal frameshifting (-1 RF) to regulate the expression ratio of Gag to Gag-Pol, which is critical for the production of infectious virion particles. A stem-loop RNA structure is one of essential components of -1 RF site of HIV-1, and its stability is important in maintaining -1 RF efficiency. Thus, small molecules interacting with high selectivity with HIV-1 RNA stem-loop might alter -1 RF efficiency and have potential to be developed as anti-HIV agents.

To identify small-molecule ligands for HIV-1 RNA stem-loop, a structure-based virtual screening was conducted. A Unity 3D search of the ZINC database and the in-house database including synthetic and natural product compounds was performed to select a primary focused compound library. The pharmacophore for Unity was determined based on the NMR solution structure of HIV-1 RNA stem-loop in complex with known ligand RG501. Docking screening of the focused library was done by using automated docking programs, such as AutoDock_vina and DOCK6.4 with Amber GB/SA scoring function. Through the analysis of virtual screening results based on the docking score and docking poses, the final candidate compounds were selected, and their effects on HIV-1 -1 RF efficiency were tested by in vitro and cell-based -1 RF assay.

P108**In Silico Identification of Agonists for Free Fatty Acid Receptors GPR40 and GPR120**

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Free fatty acid receptors (FFARs), rhodopsin-like subfamily G protein-coupled receptors, transport signals from extracellular free fatty acids, which triggers the release of hormones involved in many diseases, including type-2 diabetes, obesity, and inflammation. Among FFARs, GPR40 and GPR120 have been shown to be activated by medium- and long-chain fatty acids (FAs). GPR40–FA complex stimulates glucose-mediated insulin secretion, whereas GPR120 activates FA-stimulated GLP-1 release in L-cell. Therefore, GPR40 and GPR120 agonists have become attracted as novel therapeutic candidates for the treatment of metabolic disorders.

To identify novel agonists for GPR40 and GRP120, we built homology models based on the X-ray structures of beta-2-adrenergic receptor-Gs protein complex (PDB: 3SN6) and beta-2-adrenergic receptor (PDB: 2HR1). Built models were optimized through the docking analysis of known ligands. To select candidate compounds, the structure-based virtual screening of chemical database (Zinc DB) and in-house database (including natural product and synthetic compounds) was performed. For docking screening, both Surflex-dock and FlexX (Sybylx1.3, Tripos) program were used, and the commonly high-ranked compounds from two docking output hitlists were selected as candidate GPR40 and GRP120 agonists.

P109**Identification of SUCNR1 (GPR91) Agonists by Screening of a SOSA Library**Julien Hanson,^[a,b] Julie Gilissen,^[a,b] Bernard Pirotte^[a]

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Succinic acid is a metabolic component which takes part in the Krebs cycle, also termed citric acid cycle. It has been recently described as the cognate agonist for the orphan receptor SUCNR1 (GPR91).^[1] This receptor belongs to the G protein-coupled receptor family (GPCR), the largest class of membrane receptors characterized by seven transmembrane domains. GPCRs are involved in many physiological functions and represent 30 % of targets for currently marketed drugs. Several studies have identified a role for succinic acid as a marker of cellular ischemic stress to adjacent tissues through its receptor. The activation of SUCNR1 can induce angiogenesis, release of renin, hematopoiesis and enhancement of immunity.^[2–5] Besides, succinic acid has been shown to induce platelet aggregation.^[6] Nevertheless, the proper identification of SUCNR1 roles is limited

by the lack of small molecule pharmacological tools. The aim of this project is to identify active molecules that could serve as lead for the development of SUCNR1 modulators and thus validate the potential roles of this protein and to understand its physiological functions. Therefore, we used a luciferase-based pharmacological assay (GloSensor™ cAMP Assay, Promega)^[7] to measure cAMP levels in order to perform the screening of a selective optimization of side activities (SOSA) library, consisting of 1250 active compounds (SIGMA Lopac®).^[8] We selected five hits that presented agonist activity at the receptor and confirmed their potency with secondary evaluations. These structures will be used as template for the generation of a lead compound and further optimization of activity at SUCNR1.

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P110**Biological Activity Profiling as a Tool for Virtual Screening**Vladimir Poroikov, Dmitry Filimonov,
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Based on the freely available information about biologically active compounds (PubChem, ChEBI, ChemSpider, DrugBank, etc.), new computational tools for biological activity estimation have been developed. The applied methods vary widely from the relatively simple pairwise chemical similarity assessment to more sophisticated ligand-based or target-based approaches.

Our group published the first study describing an approach to provide chemists with information about the most relevant targets/assays for their compounds,^[1,2] and additional computational tools with similar functionality have been developed in other labs more recently as well.

Open access web services for biological activity profiling (e.g., <http://sea.bkslab.org/>, <http://cpi.bio-x.cn/drar/>, <http://bioinformatics.charite.de/superpred/>, <http://pharmaexpert.ru/passonline>) employ both target-based and ligand-based drug-design approaches. They use different mathematical algorithms and chemical structure description and prediction is provided for various biological endpoints. No systematic comparison of the accuracy and predictability of these web services has been performed yet. Therefore, we have analyzed the relative predictive power of the available services to predict the biological activity profiles using new pharmaceuticals approved by US FDA in 2011^[3] as a case study.

Accuracy of prediction for both known main & pharmacological side effects and interaction with molecular targets will be reviewed. Possibilities for increasing the accuracy and predictivity using consensus prediction with several computational methods will be explored. Applications of successful in silico bioactivity prediction in collaborative drug discovery projects will be discussed in detail. This will include the authors' own experience^[4-6] as well as some important examples taken from literature. Finally, we will discuss the prospects and limitations of using web services for bioactivity prediction in pharmaceutical research and development.

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P111

Decrease of Oxidative Stress in Idebenone-Treated Friedreich's Ataxia Patients after Oral Tocotrienol Supplementation

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In this work, we investigated white blood cell gene expression of SOD-1, SOD-2, catalase, GPX-1, GSR and GSTM-1; plasma content of GSH and GSSG; plasma oxygen radical absorbance capacity; amount of plasma carbonylated proteins; urinary levels of hexanoyl-lysine adduct; lipid composition of erythrocyte membranes.

Oxidative stress is always associated with Friedreich's ataxia (FRDA),^[1] also accompanied by impaired mitochondrial functions.^[2] Patients are currently treated with idebenone, a CoQ10 analogue, believed effective in view of its ability to counteract free radical damages.

Vitamin E is known to be effective on oxidative-stress-related pathologies,^[3] taking into account our experience in the field of the class of natural vitamin E "tocotrienol", we have started the present investigation in order to develop a model useful to investigate the efficacy of a tocotrienol-based approaches on oxidative stress damage protection. A mixture (OXI-3 internal reference name) of enantiomerically pure tocotrienols (alpha, beta, gamma and delta) has been selected and tested in patients monitoring the above-reported different biochemical parameters. The pilot investigation was conducted on five young FRDA patients who assumed OXI-3 (equivalent to 5 mg/kg/day) for two months. The wide array of different markers consistently pointed to the presence of oxidative stress in FRDA patients, despite the fact that the idebenone therapy had not been discontinued. However, even a two-month, low-dose tocotrienol supplementation led to the decrease of oxidative stress indexes and to parameter values that approached those of healthy controls. Moreover, there are evidences that a longer tocotrienol treatment may be more effective in reducing oxidative stress.

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P112

The Application of Heteroaromatic Thiosemicarbazones in Cancer Treatment

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Iron, due to its unique biochemical and biophysical properties is present in the most important processes within human cells. The transportation of oxygen and its presence in complex proteins, such as transferrin and ferritin, can be used to highlight the role of iron. It is proven that most of cancer cells have a higher requirement for iron than normal cells as they rapidly proliferate. Hence, iron metabolism is altered within these cells. This fact is reflected by higher number of Tf receptors on their cell surface, mediating a high rate of iron

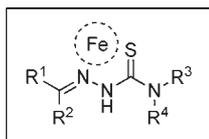
uptake. Therefore, depleting iron from rapidly dividing cancer cells through the implementation of iron chelators deprives these cells of the DNA precursors necessary for replication.

Since early 1950s thiosemicarbazones (TSC) are described as a class of compounds with a wide spectrum of biological properties. Due to their easy preparation and purification heterocyclic thiosemicarbazones are interesting medicaments with pharmaceutical applications (antibacterial, antiviral, antifungal activities). Furthermore, TSC can be perceived as a convenient *N,N,S*-donor ligands, creating various metal complexes.

All compounds were synthesized in microwave reactor (CEM-DISCOVERY®) and the purity of final products was determined by HPLC. The structures of final compounds were confirmed by NMR spectroscopy and HRMS spectroscopy.

Novel iron chelators based on thiosemicarbazone moiety have been synthesized and tested for antiproliferative activity. They were found to be active against HCT116 p53+/+ and p53-/- and SK-N-MC cancer cells (nanomolar cytotoxicity). Moreover, the ability to induce cellular iron release and inhibit iron uptake from the iron binding protein, transferrin, was at the same level that most active iron chelator Dp44mT.

The antiproliferative activity of the tested compounds was higher than DFO but lower than Dp44mT. However, several compounds have demonstrated high chelation efficiency in terms of mobilizing cellular iron and preventing iron uptake from Tf in the same level as Dp44mT. These preliminary results have shown us a high potency of synthesized compounds for inhibitions cancer cell lines. Thus, further investigations of these compounds should be conducted.



P113

New Peptidomimetic Inhibitors of the West Nile Virus NS2B-NS3 Protease

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West Nile virus (WNV) is a mosquito-borne flavivirus, which was first identified in the West Nile part of Uganda and has spread later to Asia, America and Europe. The majority of the infected humans shows no symptoms but may develop a mild flu-like illness. A small number of infected people, mainly children and the elderly, develop fatal meningitis or encephalitis leading to a mortality rate of around 10%. Despite increasing demand, there is no specific treatment of WNV infections available, so far.

A potential target for the treatment of WNV infections could be the viral NS2B-NS3 protease, which is essential for cleaving the WNV polyprotein and forms various mature viral proteins. The NS3 protein contains a serine protease domain, which cleaves their substrates preferentially at the C terminus of two basic amino acids. A well-established approach for the design of substrate-analogue inhibitors of proteases, which cleave their substrates after a basic residue, is the incorporation of decarboxylated arginine mimetics in P1 position. This strategy was used for the design of inhibitors for various trypsin-like serine proteases and furin-like proprotein convertases. Very recently, first peptidomimetic agmatine derivatives with inhibition constants around 2 μM have been described by a group from Singapore.^[1]

We have prepared new analogues within this inhibitor type. The replacement of agmatine by suitable cyclic P1 moieties in combination with substitutions at the P4 residue provided several compounds with inhibition constants <0.2 μM . All derivatives were characterized as classical competitive inhibitors. Molecular modeling revealed similar key interactions as found previously in crystal structures of the WNV protease with covalently bound arginal-derived inhibitors. Such protease inhibitors could be new lead structures for the development of potential drugs for the treatment of WNV infections.

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P114

Development, Validation and Application of an LC-ESI-MS/MS Quantification Method for a Potential GAT4 Marker

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The γ -aminobutyric acid (GABA) transporter subtype 4 (GAT4, solute carrier 6 (SLC6) a11) resembles a promising drug target in the development of new drug candidates for diseases like epilepsy, morbus Parkinson's disease, morbus Alzheimer's disease and anxiety. Mass spectrometry based binding assays (MS binding assays) employing a non labelled marker addressing GAT4 could be assumed to facilitate the search for potent GAT4 inhibitors as recently demonstrated for other neurotransmitter transporters.^[1,2] So, our aim was to develop a sensitive quantification method for a potential GAT4 marker as a prerequisite for MS binding assays. From the so far most potent GAT4 inhibitors, we selected DDPM-1007 ((*R,S*)-1-[4,4,4-tris(4-methoxyphenyl)but-2-en-1-yl]piperidine-3-carboxylic acid-HCl), a carba analogue of (*S*)-SNAP-5114 ((*S*)-1-(2-[tris(4-methoxyphenyl)methoxy]ethylpiperidine-3-carboxylic acid) due to its enhanced chemical stability.^[3]

Using a 50 mm x 2 mm C8 column in combination with a mobile phase composed of 10 mM ammonium bicarbonate buffer pH 8.0 and acetonitrile (60:40, v/v) at a flow rate of 450 $\mu\text{L}/\text{min}$, DDPM-1007

could be analyzed in the positive MRM mode (m/z 502.5→265.4) by means of an API 5000 triple quadrupole mass spectrometer within a chromatographic cycle time of 3 min. [$^2\text{H}_3$]DDPM-1007 containing three [$^2\text{H}_3$]methoxy moieties was synthesized as isotopically labelled internal standard in order to compensate for potential matrix effects resulting from binding samples. Thus, DDPM-1007 could be quantified in a range from 100 pM to 10 nM in samples obtained from respective binding experiments without any sample preparation. The established quantification method met the requirements of the US FDA guidance for bioanalytical method validation concerning linearity, intra- and inter-batch accuracy. Applying this LC-MS/MS method to preliminary MS binding assays employing membrane preparations obtained from a stably mGAT4 expressing HEK293 cell line and DDPM-1007 as non-labelled GAT4 marker specific binding of DDPM-1007 at GAT4 could be unambiguously detected.

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P115

Novel Dibenzoxepine and Benzosuberone p38 α MAP Kinase Inhibitors: Extending Interactions to the Deep Pocket / from Type I to Type II Inhibitors

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The p38 mitogen-activated protein kinase (p38 MAPK) plays a key role in the pathogenesis of many inflammatory and autoimmune diseases, for example rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD) and psoriasis. In general there are three different types of p38 MAPK inhibitors: type I, type II and type III inhibitors. Type I inhibitors are ATP competitive. Type II inhibitors use an extra hydrophobic pocket ("deep pocket"), which is only available when the activation loop changes its conformation. Type III inhibitors bind in an allosteric region of the enzyme.^[1,2]

Recently, we described dibenzosuberone^[3] and dibenzoxepine^[4,5] compounds as highly selective type I inhibitors. The main goal of this project is to synthesize and evaluate new dibenzosuberone and dibenzoxepine analogues designed to reach the deep pocket of p38 MAPK to combine both extreme selection of this class of compounds with the slow off-kinetic of type II inhibitors.

The employed strategy to design new compounds as type II inhibitors was based on the insertion of hydrophobic aromatic side chains or hydrophilic groups on dibenzoxepine scaffolds, aiming to identify which position and substituent is the most effective on reaching the "deep pocket", finally contributing to the increase of affinity.

The designed dibenzoxepine compounds were synthesized and evaluated by an enzymatic assay to determine their ability to inhibit the p38 α MAPK through the quantification of substrate phosphorylation.^[6]

The insertion of different hydrophobic and hydrophilic groups resulted in novel benzosuberone and dibenzoxepines derivatives designed as p38 α MAPK inhibitors with IC₅₀ values down to 30 nM.

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P116

Design and Synthesis of New Cyanothiophene Inhibitors of MurF

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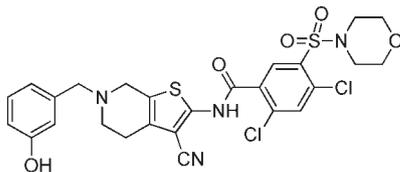
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Peptidoglycan is an essential component of the bacterial cell wall and enzymes involved in its biosynthesis represent validated targets for antibacterial drug discovery. Mur ligases (MurC to MurF) are intracellular ATP-dependent enzymes that catalyze the sequential

addition of L-Ala, D-Glu, *meso*-DAP or L-Lys, and D-Ala-D-Ala dipeptide to UDP-Mur *N*-Ac to form UDP-Mur *N*-Ac-pentapeptide. MurF catalyzes the ultimate addition of D-Ala-D-Ala to the nucleotide precursor UDP-Mur *N*-Ac-L-Ala-D-Glu-*meso*-DAP (or L-Lys). Since it has no human counterparts, this enzyme represents an attractive target for the development of new antibacterial drugs.^[1]



IC₅₀ (MurF from *S. pneumoniae*) = 0.418 μM
 IC₅₀ (MurF from *E. coli*) = 81 μM
 IC₅₀ (MurF from *S. aureus*) = 91 μM
 MIC (*S. pneumoniae* R6) = 16 μg/mL

Using recently published Abbott inhibitors of MurF from *Streptococcus pneumoniae* as a starting point,^[2,3] we have designed and synthesized a series of structurally related cyanothiophene derivatives and investigated their inhibition of MurF enzymes from different bacterial species. Structural modifications of parent compounds resulted in a series of nanomolar inhibitors of MurF from *S. pneumoniae* and micromolar inhibitors of MurF from *Escherichia coli* and *Staphylococcus aureus*. Some of the inhibitors also exhibited antibacterial activities against *S. pneumoniae* R6 bacterial strain. These findings represent an excellent starting point for further optimization towards effective novel antibacterial drugs.

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P117

N-Substituted Phthalazinones as Potential Dual Inhibitors of Cholinesterase and Monoamine Oxidase

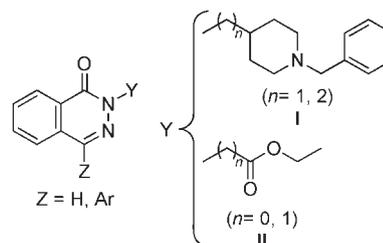
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The proposal that multitarget ligands could be very useful for treatment of neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases, has encouraged medicinal chemists to develop drugs with two or more complementary biological activities.^[1] Thus, taking into account that cholinesterase (AChE and BuChE) and monoamine oxidase (MAO-A and MAO-B) are enzymes that modulate biochemical changes related to this kind of disorders,^[2] in recent years was developed ladostigil, a new bifunctional drug that contains two pharmacophores, the carbamate group of rivastigmine (AChE inhibitor) and the propargylamine group of rasagiline (MAO-B inhibitor). Ladostigil inhibits both ChE (AChE and BuChE) and brain MAO (MAO-A and MAO-B).^[3]

Looking for new mixed ChE/MAO inhibitors, we have designed novel families of hybrid compounds of structures I and II. These molecules combine *N*-benzyl piperidine or carbamate fragments with the hydrazido moiety, including pharmacophoric features of donepezil or rivastigmine (two potent AChEI) and isocarboxazid (a nonselective MAOI).



The *N*-benzyl piperidine derivatives were synthesized in four steps using as starting materials the adequate 2*H*-phthalazin-1-ones and two commercially available *N*-Boc-protected 4-hydroxyalkylpiperidines. First, the hydroxyalkyl derivatives were transformed into the 4-bromoalkylpiperidines; then, the phthalazinones were treated with sodium hydride and the appropriate bromoalkyl derivative in DMF to give the corresponding 2-(*N*-Boc-4-piperidinylalkyl)phthalazin-1-ones, which, after acid hydrolysis (HCl) of protecting group, were converted into the desired compounds by reaction with benzyl bromide in the presence of sodium hydride.

The carbamate analogues were synthesized in one step starting from the corresponding 2*H*-phtalazin-1-one by reaction with sodium hydride and ethyl bromoacetate in DMF (*N*-ethoxycarbonylmethyl derivatives) or by treatment with ethyl chloroformate and triethylamine in DCM (*N*-ethoxycarbonyl analogues).

The eight synthesized compounds were evaluated as cholinesterase (hAChE and hBuChE) and monoamine oxidase (hMAO-A and hMAO-B) inhibitors. The results of this biological study will be reported.

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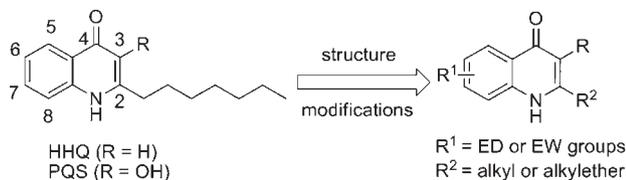
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P118

Anti-infectives with Novel Mode of Action: Discovery of the First Antagonists of PQS to Interrupt *P. aeruginosa* Cell-to-Cell Communication

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Pseudomonas aeruginosa coordinates group behaviors via a cell density dependent cell-to-cell communication system known as quorum sensing (QS).^[1] It employs a characteristic *pqs* QS system that functions via the signal molecules PQS and its precursor HHQ that interact with their receptor PqsR to control the transcription of virulence genes and biofilm formation. PqsR is considered as a potential target to reduce *P. aeruginosa* pathogenicity. In order to discover PqsR antagonists, a ligand-based approach was followed and HHQ and PQS derived compounds were synthesized.

To investigate agonistic or antagonistic properties, a β -galactosidase reporter gene assay in *E. coli* was established. SARs of side chain modifications and substitutions at the benzene moiety were evaluated. An *n*-heptyl chain in 2-position was found to be optimal. Importantly, introduction of strong electron-withdrawing groups like CN, NO₂ or CF₃ in 6-position of HHQ resulted in the first competitive antagonists (IC₅₀ values of 259 nM, 51 nM and 54 nM), while HHQ analogues with the same substituents in 7- or 8-position or other substituents and all PQS derivatives were moderate to weak agonists. Direct evidence for the binding of a selected antagonist (6-CN HHQ) to PqsR was provided by surface plasmon resonance (SPR) biosensor experiments. In pyocyanin assay, which functions as a biologic readout for virulence expression, 6-CF₃ HHQ reduced pyocyanin production in *P. aeruginosa* by 74% (3 μ M).^[2] Water solubility of antagonists was improved by introduction of O into the side chain or CONH₂ into 3-position. The discovery of the first antagonists of PqsR provides a promising starting point for the development of a new anti-infective strategy.

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P119

Development of a p38 δ MAPK ELISA Assay for Quantitative Determination of Inhibitor Activity

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p38 Mitogen-activated protein kinases (MAPKs) are members of a larger group of serine/threonine protein kinases contributing in a variety of cellular processes such as gene expression, mitosis, differentiation, cell survival/apoptosis and biosynthesis/release of pro-inflammatory cytokines.^[1] The role of p38 α isoform is widely investigated in many inflammatory diseases like rheumatoid arthritis (RA). Activated rheumatoid arthritis synovial fibroblasts (RASFs) can be considered as key cells in the development of RA, since they mediate the most relevant pathways of joint destruction.^[2,3] The activation of p38 δ MAPK in RASFs by a cytokine-independent pathway leads to the expression of matrix metalloproteinases, e.g. MMP-1 and MMP-3, which contribute to the destruction of articular cartilage and bone.^[3,4] All four isoforms (α , β , γ and δ) of the p38 MAPK have been detected in the RA synovial tissue, but at the site of invasion and bone destruction p38 δ MAPK occurs predominantly and for this, the participation of p38 δ MAPK becomes more and more evident.^[2,5]

To identify inhibitors of p38 δ MAPK, we developed a direct 96-well plate ELISA assay for the purpose of routine inhibitor screening. The activity of p38 δ MAPK after incubation with a candidate inhibitor is measured by the phosphorylation degree of activation transcription factor 2 (ATF-2). The phosphorylated ATF-2 is directly detected by a monoclonal peroxidase conjugated antibody. ATF-2 is a natural substrate of the p38 MAPKs, and its phosphorylation is inversely correlated with the inhibitor potency. Based on already successfully established ELISA assays for p38 α MAPK and JNK3, the advantages of this assay are its accuracy, easy handling, rapidness and the avoidance of using radioisotopes.

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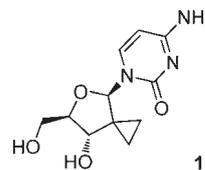
2'-Deoxy-2'-spirocyclopropyl Cytidine: A New and Selective Inhibitor of HCV NS5B Polymerase

Tim H. M. Jonckers, Tse-I Lin, Christophe Buyck, Sophie Lachau-Durand, Koen Vandyck, Lili Hu, Jan Martin Berke, Leen Vijgen, Lieve L. A. Dillen, Maxwell D. Cummings, Herman de Kock, Magnus Nilsson, Christian Sund, Christina Rydegård, Bertil Samuelsson, Åsa Rosenquist, Gregory Fanning, Kristof Van Emelen, Kenneth Simmen, Pierre Raboisson

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The current standard therapy for hepatitis C virus (HCV) infection is hampered by limited efficacy, in particular against the genotype 1 virus, and a range of side effects. In this context of high unmet medical need, novel more efficacious drugs targeting HCV nonstructural proteins are of key interest. We have identified 2'-deoxy-2'-spiro-cyclopropyl cytidine (**1**) as a new inhibitor of the HCV NS5B RNA-dependent RNA polymerase, displaying an EC₅₀ value of 7.3 μ M measured in the Huh7-Rep cell line containing the ET replicon clone. Computational results indicated high structural and electronic similarity between **1** and related HCV inhibiting nucleosides. In this communication, we will discuss the design, synthesis and pharmacokinetic properties of **1** and some prodrug derivatives thereof.



P121

In Vitro Study on the Biotransformation of a Skepinone-L-Like p38 Mitogen-Activated Kinase Inhibitor

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The p38 mitogen-activated kinases (MAPK) are validated targets for many inflammatory diseases, e.g. rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD) and psoriasis,^[1] whereas in cancer selective multikinase inhibitors are common treatments.^[2] For chronic treatment of inflammatory diseases, highly selective p38 α inhibitors (e.g., Skepinone-L)^[3] could be beneficial.

As metabolism is crucial in early drug discovery stages, we investigated basic metabolism pathways of Skepinone-L derivative (3-((2,4-difluorophenyl)amino)dibenzo[*b,e*]oxepin-11(6*H*)-one. This is a new potent and selective inhibitor for p38 α MAPK as described above. In the present study, biotransformation is observed after incubation with male and female Wistar rat and Sprague–Dawley rat microsomes using LC-MS/MS. The formation of the predominant metabolite was characterized in more detail using liver microsomes from various rat species as well as single cytochrome P-450 isoforms in order to identify the metabolic active isoform. Furthermore it was possible to quantify the developing metabolite by internal and external calibration. Major pathways of metabolism are dehalogenation and hydroxylation.

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P122

Peroxide-Based Hybrid Compounds for Multistage Targeting of Malaria Parasites

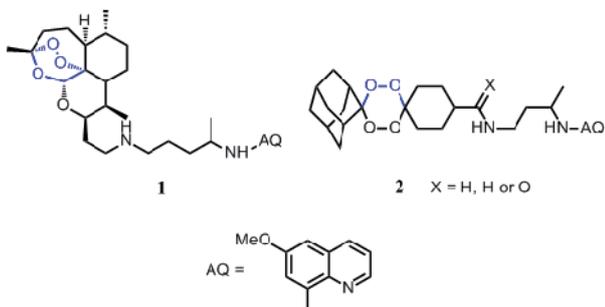
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Malaria eradication requires novel prophylactic and therapeutic approaches targeting the obligatory liver stage and the erythrocyte-infecting parasites.^[1] Unfortunately, there are no drugs capable of killing simultaneously the blood- and liver-stage of malaria parasites. An alternative approach is to link two pharmacophores, each one targeting a specific stage of the parasite's life cycle, in a single molecule called hybrid drug.



Following our initial report on primaquine-artemisinin hybrid compounds,^[2] we now report on the development of hybrid molecules encompassing 8-aminoquinoline and hemisynthetic endoperoxide-based (**1**) or synthetic tetraoxane-based (**2**) pharmacophores, to convey activity against both the liver and the blood stages of the parasite. These compounds displayed excellent in vitro activity against blood stage infection by *P. falciparum* and liver stage infection by *P. berghei*. The metabolism was studied in rat liver microsomes, revealing that hybrids **1** and **2** display half-lives for degradation ranging from 7 to approximately 50 h. These results strongly suggest that the design of hybrid compounds represent an attractive approach to develop antimalarial agents capable of interfering with blood- and liver-stage malaria parasites.

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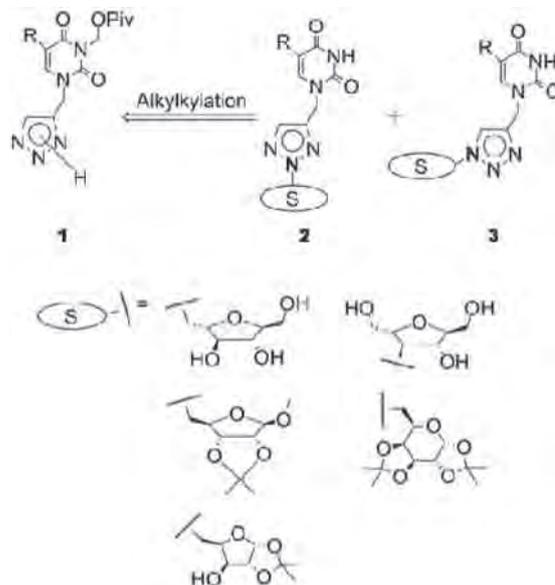
P123

Convenient Approach to Isomeric Nucleoside 1,2,3-Triazoles

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Nucleoside analogues with a 1,4-disubstituted-*NH*-1,2,3-triazole spacer between a nucleobase and a sugar or a sugar mimic have recently attracted particular attention owing to their interesting biological or materials properties.^[1] Our approach to isomeric nucleoside 1,2,3-triazoles **2** and **3** involved an alkylation of *NH*-1,2,3-triazole **1** with a sugar tosylate or epoxide. Results of the research *N*(2)/*N*(1)-selectivity of the triazole alkylation process will be presented.



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P124

Novel Promising Therapeutics for the Treatment of Osteoporosis: Highly Potent and Selective 17 β -Hydroxysteroid Dehydrogenase Type 2 (17 β -HSD2) Inhibitors

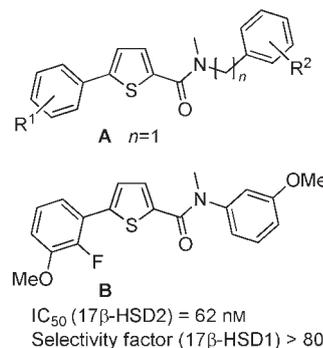
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Reduction of the estrogen levels in aging women often leads to osteoporosis. Estrogens like estradiol (E2) and androgens like testosterone (T) are known to be involved in bone maintenance,^[1,2] inhibiting bone resorption in the osteoclasts and inducing bone formation in the osteoblasts, respectively. Replacement estrogen therapy is efficient in the treatment of this disease but cannot be applied as it leads to severe adverse effects. Administration of a drug, which could increase E2 and T levels in bone cells, could become an alternative therapy to bisphosphonates and selective estrogen receptor modulators (SERMs) for the treatment of osteoporosis.

A promising approach to increase the level of E2 and T in bone might be the inhibition of the enzyme 17 β -hydroxysteroid dehydrogenase type 2 (17 β -HSD2), which is present in bone cells. This protein catalyzes the conversion of the highly potent E2 and T into less active estrone (E1) and androstenedione, respectively. Potent and selective inhibitors of this enzyme are required to prove the validity of this concept and of the target. Selectivity should be achieved towards 17 β -HSD1, which is responsible for the reverse reaction, i.e., transformation of E1 into E2 and towards the estrogen receptors (ER) α and β .

A ligand-based rational drug design approach led to the development of the previously described benzylthiophene amides (compound **A**, $n=1$) as inhibitors of 17 β -HSD2.^[3] Structural optimisation has been performed by variation of the linker size ($n=0$ or 2) and led to the identification of compound **B** as new highly potent inhibitor of the target enzyme with an IC_{50} value of 62 nM and displaying good selectivity toward 17 β -HSD type 1 (selectivity factor >800) as well as no binding affinity to ER α and β . In order to identify the best appropriate species for a proof of principle, the most potent and selective derivatives were tested on mouse, rat and monkey enzymes. The new designed structures, their activity and selectivity profiles as well as their potencies toward 17 β -HSD2 from different species will be presented.



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P126

Tetrasubstituted Imidazoles as a New Template for Inhibitors of the p53–MDM2 Interaction

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The p53 tumor-suppressor protein plays a key role in the control of cellular integrity. Loss of function of the p53 gene by mutations or deletions is observed in almost 50% of all human cancer tissues.^[1] In other cancer tissues, still expressing the wild-type form, the normal function of p53 is altered by overexpression or amplification of MDM2 (or HDM2), the main negative regulator of the tumor suppressor. In this setting, MDM2 mainly functions as a p53 specific ubiquitin ligase, which, by binding to the N-terminal transactivation domain of p53, triggers its proteasomal degradation. Cancerous cells having elevated MDM2 levels are thus protected against p53 dependent apoptosis and cell cycle arrest mechanisms.^[2]

To restore normal p53 function in such tumor cells, one can envisage to disrupt the p53–MDM2 interaction by small molecules having high affinity for the p53 binding pocket of MDM2.^[3] This attractive therapeutic concept has raised a lot of interest in anticancer drug research and some molecules exerting an antiproliferative activity by this mechanism have entered clinical evaluation.^[4]

Several years ago, we initiated an effort in this direction by the identification of a very potent octapeptide inhibitor of the p53–MDM2 interaction incorporating non-natural amino acids.^[5] This

octapeptide was designed on the basis of the available crystal structure of MDM2 in complex with a 15-mer peptide derived from the natural sequence of p53.^[6]

Since that time, we have pursued this effort by the search for non-peptide inhibitors of this critical protein-protein interaction showing cellular activity.

Along this line, we have recently reported the identification of a new promising p53–MDM2 interaction inhibitor chemotype by structure-based design.^[7] The design concept relied on a peculiar topological feature of the p53 binding pocket of MDM2.

Following the same concept, we have discovered a second class of potent inhibitors. We report here the design of these new inhibitors based on a tetrasubstituted imidazole ring as core structure. Their optimization towards compounds showing significant cellular antiproliferative activity is also presented.

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P127

Discovery of Novel 2-Benzamidoacetic Acid Derivatives as PTP1B Inhibitors and Antihyperglycemic Agents

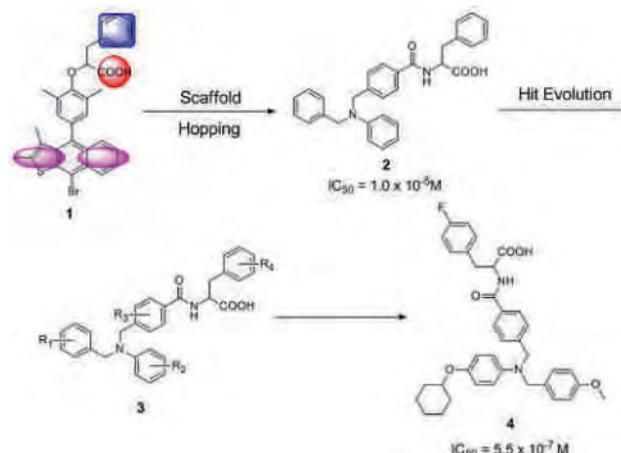
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Protein tyrosine phosphatase (PTP) 1B is an emerging therapeutic target for type 2 diabetes. However, the highly cationic nature of its active site makes PTP1B a challenging target for drug discovery.^[1] We report herein the discovery of novel PTP1B inhibitors with in vivo antidiabetic effects by exploiting a molecular design strategy of pharmacophore-oriented scaffold hopping.

The known PTP1B inhibitor, erlotinib (1),^[2] was used as a chemical template, and a composite pharmacophore with four features was derived from the binding mode of 1. Subsequent pharmacophore-oriented scaffold hopping led to the discovery of novel 2-benzamidoacetic acid derivative 2 as a hit compound with evident PTP1B inhibitory activity. Hit evolution guided by molecular docking was

performed, and about 50 compounds with the general formula 3 were synthesized and evaluated. Among them, seven compounds were recognized as potent PTP1B inhibitors with IC₅₀ values of 10⁻⁷ M level. One of the most active compounds, 4, was further evaluated with DIO insulin-resistant mice, and exhibited significant in vivo antidiabetic activity.



In summary, after two turns of iterative design, synthesis and evaluation, a new lead with in vivo antidiabetic activity has been discovered and preliminary structure-activity relationships (SAR) revealed. The lead structure possesses intellectual property and is more chemically available than known PTP1B inhibitors. The current results implicated the effectiveness of molecular design and provided constructive clues for further optimization.

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P129

Structure-Based Design of Novel Aryl Aminopyridine Derivatives as Potential Cyclin-Dependent Kinase 7/9 Inhibitors

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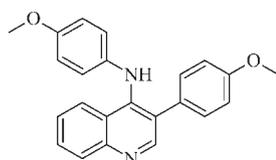
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Cyclin-dependent kinases (CDKs) 7 and 9 are protein kinases involved in the transcriptional regulation of cell cycle progression. They present potentially important targets for novel therapeutics in oncology, virology and cardiology.^[1,2] Our group recently reported the synthesis of 16 novel aryl aminopyridine derivatives,^[3] 12 of which were found to possess antiproliferative activity presumably due to the interaction with several important protein kinases (unpublished results). Here, we present a structure-based approach to the modification of these novel aminopyridines aimed at improving their activity and selectivity for CDKs 7 and 9, respectively.

The BindingDB was searched to identify small-molecule ligands of human CDK 7 and 9 with known binding affinities. A total of 26 CDK7 and 23 CDK9 ligands were selected, and their structural similarity to the investigated aminopyridines was evaluated using OpenEye ROCS. To elucidate molecular interactions with human CDKs 7 and 9, these ligands and the 12 aminopyridines were subsequently docked into the corresponding crystallographic structures using AutoDock 4.0.

ROCS analysis revealed that there is a significant degree of structural similarity between the studied aminopyridines and some of the known CDK 7 and 9 ligands. Potential of these compounds to interact with CDKs 7 and 9 was further supported by the docking results. Binding modes and energies seem to suggest these aryl aminopyridines bind more favorably to the ATP-binding site of CDK7 than that of CDK9. One of the aminopyridines (pictured) was determined to show the highest affinity for both proteins. Comparison of its binding mode to that of potent CDK 7/9 inhibitors revealed a significant potential for improvement of affinity through structural modifications that are further discussed. The studied scaffold seems to present a promising basis for the design of novel potent CDK7 inhibitors.



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P130

Novel Oxycarbonylselenoesters as Selective Anticancer Agents

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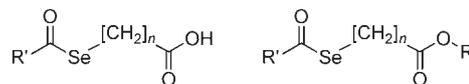
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Selenium is a trace element whose derivatives fulfil important biological functions, and have been widely studied in cancer prevention and chemotherapy due to their roles as antioxidant and antiproliferative agents.^[1] Hence, our group has explored recently the anticancer properties of different selenoderivatives. In this research, 26 carboxymethylselenoesters were synthesized and biologically evaluated,^[2] and, although most active ones had noteworthy antiproliferative values in the cancer cell line (PC-3), overall activity was below expected values. In order to improve the biological activity, 15 methyl, *tert*-butyl and phenyl carboxylic esters of the most active carboxymethylselenoesters were synthesized. As preliminary results in prostate cancer cells (PC-3) showed that methyl esterification enhances the biological effect,^[3] nine selected derivatives were studied in more depth at the Université Paris Descartes against a panel of eight cell lines using the Crystal Violet method to avoid redox interferences with selenium atoms.



R=methyl, *tert*-butyl, phenyl

n=1,2

R'=benzyl, phenyl, 2-chlorophenyl,
4-chlorophenyl, 3,5-dimethoxyphenyl,
3,4,5-trimethoxyphenyl, 2-thienyl

Results indicate that selenoesters are more active in cancer cell lines HT-29 (colon), MCF-7 (breast) and A549 (lung) than in HepG2 (liver) and OVCAR-3 (ovary). It is observed that six of the nine selenoesters tested have interesting IC₅₀ values below 10 μM and an activity comparable with known anticancer drugs such as taxol and doxorubicin in at least three of the five human tumoral cell lines assayed; showing three derivatives selectivity indexes above three in comparison with nontumoral embryo cells, HUVEC. In the two murine cell lines tested, results are not as conclusive as in the human cells for compounds being less active and selective, although two derivatives are in the same order as the reference

chemotherapy drugs. In conclusion, the IC₅₀ values point towards oxycarbonylselenoesters as potential novel selective anticancer agents.

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P131

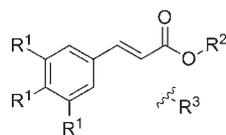
Cytotoxic Activity of Cinnamic Acid Derivatives on Human Cancer Cell Lines

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R¹ = H or OMe

R² = H, Ph, Bn, 4-CN-Ph, 3-PhOPh, cyclohexyl, 2-phthalimidooethyl

R³ = 4-methylpiperidine-1-yl, (S)-benzyl 2-aminopropanoate

Cinnamic acid derivatives are widely distributed in plant material and possess a broad spectrum of biological activities. In the past few years, several reports about cytotoxic and antitumor activities of cinnamic acid derivatives have been published.^[1] The aim of our work was to investigate the cytotoxic effects of selected cinnamic acid esters and amides. The MTT test was used for determination of cytotoxic effects on four different cancer cell lines: estrogen-receptor-positive breast cancer (MCF-7), myelogenous leukemia (K562), malignant melanoma (Fem-x), and human cervix adenocarcinoma (HeLa) cells. To obtain the information about selectivity, normal human cells (peripheral blood mononuclear cells [PBMCs]) were used with or without stimulation by plant lectin phytohemagglutinin, the known stimulator of proliferation of lymphocytes. The compounds tested showed significant cytotoxicity on all cancer cell lines (IC₅₀ values between 42 and 166 μM). Furthermore, selectivity of these cytotoxic effects on the malignant cell lines versus the PBMCs was also seen, especially when a cyano group was present on the aromatic ring of the alcohol or amine part. The additional study on cell cycle phase distribution of tested cell lines indicated that novel cinnamic acid derivatives inhibit cell growth by selective induction of cell death and disruption of cell cycle. Therefore, the

tested cinnamic acid derivatives represent a good starting point for further derivatization and development towards antineoplastic drug candidates.

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P132

The Quinoline Imidoselenocarbamate EI201 Blocks the AKT/mTOR Pathway and Targets Cancer Stem Cells Leading to a Strong Antitumor Activity

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Methylimidoselenocarbamates have previously proven to display potent antitumor activities.^[1,2] In the present study, we show that these compounds act as multikinase inhibitors. We found that the most effective compound, quinoline imidoselenocarbamate EI201, inhibits the PI3K/AKT/mTOR pathway, which is persistently activated and contributes to malignant progression in various cancers.^[3] EI201 blocked the phosphorylation of AKT, mTOR and several of its downstream regulators (p70S6K and 4E-BP1) and ERK1/2 in PC-3, HT-29 and MCF-7 cells in vitro, inducing both autophagy and apoptosis. EI201 also contributes to the loss of maintenance of the self-renewal and tumorigenic capacity of cancer stem cells (CSCs). 0.1 μmol/L EI201 triggered a reduction in size and number of tumorspheres in PC-3, HT-29 and MCF-7 cells and 4 μmol/L induced the elimination of almost all the tumorspheres in the three studied cell lines. In addition, EI201 suppressed almost 80% prostate tumor growth in vivo (*p*<0.01) compared to controls at a relatively low dose (10 mg/kg) in a mouse xenograft model. There was a significant decrease in the subcutaneous primary tumor [¹⁸F]-FDG uptake (76.5% reduction, *p*<0.05) and in the total tumor burden (76.8% reduction, *p*<0.05) after EI201 treatment compared with vehicle control, without causing toxicity in mice. Taken together, our results support further development of EI201 as a novel multikinase inhibitor that may be useful against cancers with aberrant upregulation of PI3K/AKT and MAPK signaling pathways.

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P133

Structure and Ligand-Based Identification of Novel Synthetic Ligands for Farnesoid X Receptor

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Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily and acts as a ligand-activated transcription factor.^[1] It is highly expressed in liver, intestine and kidney and binds to its DNA response elements as a heterodimer with retinoid X receptor (RXR). FXR regulates a large number of target genes which are involved in bile acid metabolism, lipid and glucose homeostasis. Bile acids (most active: chenodeoxycholic acid) as well as their metabolites and polyunsaturated fatty acids are known as natural ligands for FXR.

FXR became a promising target for the treatment of several diseases like non-alcoholic fatty liver disease (NAFLD) and primary biliary cirrhosis (PBC). Several synthetic ligands have been developed and led to an increased knowledge of function and role of FXR in metabolic regulation of bile acids and cholesterol as well as in inflammatory pathways within the intestine. FXR activation by synthetic ligands turned out to reduce plasma triglycerides and cholesterol as well as atherosclerotic lesions. Animal models additionally showed beneficial effects of FXR activation on insulin resistance.^[2,3] Furthermore, it was recently discovered that FXR activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease (IBD) in a mouse model.^[4]

Extensive industrial and academic research on FXR has yielded several potent ligands, such as GW-4064 and its derivatives, MFA-1, fexaramine and 6-ECDC. But besides 6-ECDC, which has advanced clinical phase II trials for NAFLD, none of the compounds has the potential to become a drug yet. This is partly due to insufficient druglikeness, poor bioavailability or toxicity of the experimental ligands. Furthermore, because of the high lipophilicity and large size of the FXR ligand binding site, the design of new ligands with drug-like properties is difficult. The development of novel modulators of FXR is therefore still a challenging topic for medicinal chemistry.^[5]

It has previously been observed that ligands for the peroxisome proliferator-activated receptors (PPAR) can also show activity at FXR.^[6] We therefore screened our in-house compound library of PPAR ligands in our Gal-4 FXR transactivation assay. Starting from one hit—HZ55^[7]—as lead structure for FXR ligands, we synthesized sev-

eral oxypropoxybenzoic acid derivatives. We observed extensive differences in pharmacological characteristics for structural minimally varying moieties like quinolin- or pyridine substituents. In addition to the synthesis of novel ligands for FXR, we are also improving our Gal-4 assay to full-length assay and developing new assay systems for nuclear receptors.

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P135

Flexible Docking Study of Transient Receptor Potential Vanilloid Subtype 1 Antagonists

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Transient receptor potential vanilloid subtype 1 (TRPV1) is a member of the transient receptor potential (TRP) and a ligand-gated nonselective cation channel superfamily. It is known to be an important therapeutic target for pain relief. TRPV1 antagonists in particular have attracted much attention as promising drug candidates to inhibit the transmission of nociceptive signals from the periphery to the CNS and to block other pathological states associated with this receptor.

Based on the dibenzyl thiourea analogue as a lead compound, the diarylalkyl amide and furan-linked amide analogues were designed and synthesized as rat TRPV1 (rTRPV1) antagonists. Using our rTRPV1 model, we performed the flexible docking study of the

tested compounds, and the results were consistent with their rTRPV1 activities. Although the binding mode of the diarylalkyl amide was not good, the furan-linked amide analogue as well as the dibenzyl thiourea fitted well into the binding site. The rigidity of B-region could contribute to the appropriate positioning of the C-region for the hydrophobic interactions.

Moreover, the 4-methylsulfonamide derivatives were designed and synthesized as human TRPV1 (hTRPV1) antagonists. The additional bulky hydrophobic group in the C-region led to a dramatic increase of the hTRPV1 activity. To investigate the structure–activity relationships, we constructed the hTRPV1 tetramer homology model and performed the flexible docking study. The tested compounds occupied the binding site very well and formed tight interactions via the hydrophobic and H-bonding interactions with the binding site residues. Furthermore, the additional hydrophobic group made another hydrophobic interaction with the hydrophobic region, composed of Met514 and Leu515. That might explain why the 4-methylsulfonamide derivative with an additional hydrophobic group showed much more potent activity.

P136

Benzimidazole and Pyridone Derivatives as New Potential Inhibitors of Phosphodiesterase

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Cyclic nucleotide phosphodiesterases (PDEs) play a major role in cell signalling by hydrolysing the ester bond of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Human PDEs comprise a family of 21 genes, the products of which fall into 11 families with as many as 60 isoforms. Due to their diversity, PDEs can selectively regulate various cellular functions in many pathologies such as cancer, inflammation, neurodegeneration, oxidative stress and so forth.^[1]

The positive inotropic effects of clinically available drugs, including the pyridone derivatives (amrinone, milrinone) and the benzimidazole derivatives (sulfamazole, pimobendane) are due to inhibition of cardiac PDE3 activity with a subsequent increase in myocardial cAMP content. Inotropic agents are indispensable for the improvement of cardiac contractile dysfunction in acute heart failure. It is important that an increase in cAMP leads not only to positive inotropic but also to positive chronotropic effects.^[2] Amrinone and milrinone act via cAMP/protein kinase A (PKA)-mediated facilitation of intracellular Ca²⁺ mobilisation. They also have a vasodilatory action, which plays a role in improving haemodynamic parameters in certain patients. The use of inotropic agents may result in Ca²⁺ overload leading to arrhythmias, myocardial cell injury and ultimately, cell death. In addition, they lose their effectiveness under

pathophysiological conditions, such as acidosis, stunned myocardium and heart failure. Pimobendan, which acts by a combination of an increase in Ca²⁺ sensitivity and PDE3 inhibition, appears to be more beneficial among existing agents but in fact offers no advantage over milrinone.^[3] Because of many disadvantages of PDE3 inhibitors, we are looking for more efficient agents. Our products contain a pyridone or benzimidazole moiety. They were synthesized by the reaction of 2-bromobenzimidazole or 6-bromo-2-pyridone with an *N*-substituted 2,3-epoxypropyl-1-amine.^[4] The obtained compounds were evaluated for their influence on phosphodiesterase activity by determination of cAMP in liver homogenate by Lance cAMP assay in time-resolved fluorescence resonance energy transfer (TR-FRET) technology. Inhibition of cAMP degradation was proportional to the inhibition of PDE by the tested compound.

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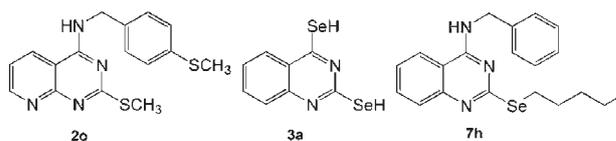
Novel Quinazoline and Pyrido[2,3-*d*]pyrimidine Derivatives Inhibit the Migration in MDA-MB-231 Cells

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The disease of cancer has been ranked as a major health burden.^[1] An important aspect of cancer treatment is the prevention of invasion and metastasis^[2] and, due to this, cell migration appears as a very promising process that could be targeted for drug development. Src and FAK kinases seem to control these processes, because an increase expression of both has been associated with more aggressive and invasive phenotypes.^[3]



One of the most promising aspects of the quinazoline and pyrido[2,3-*d*]pyrimidine rings is their activity as anticancer agents. Continuing with the investigations of our group,^[4,5] three compounds were chosen as lead compounds for investigation of their capacity to inhibit cell migration.

We have demonstrated that these compounds induce concentration-dependent inhibition of the migration of MDA-MB-231 cells. Compound **3a** inhibits the migration of these cells by 20% at 100 nM concentration and is the most potent of the derivatives tested. In this inhibition of migration, the kinases Src and FAK are not implicated, since the phosphorylation levels of these proteins are not affected following treatment.

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Identification of a Peptidic Non-ATP Competitive Inhibitor of Human CK2 by Bacterial Surface Display Library Screening

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Human protein kinase CK2 plays an important role in the genesis of cancer. Elevated CK2 activity has been associated with the malignant transformation of several tissues and serves as a prognostic marker of cancer.^[1] Up to date several CK2 inhibitors were developed. Most of them target the highly conserved ATP cavity and show a weak selectivity throughout human protein kinases.^[2] Hence, there is increasing interest in the development of inhibitors with a different mode of inhibition. The aim of the study was to find a non-ATP competitive inhibitor of CK2.

Further studies evidenced that it is possible to display an inhibitor on the surface and to label this inhibitor by the target enzyme. Furthermore, it was shown that it is possible to sort single cells labeled by a fluorophore coupled target enzyme.^[3] In this study a 12-mer library was surface displayed on *Escherichia coli* outer membrane. This library contained 6×10^5 variants and was screened with fluorophore-conjugated CK2 by flow cytometry. Single cell variants showing affinity to the CK2 holoenzyme were sorted, and the coding sequence for the binding peptide variants was revealed by DNA sequence analysis. The corresponding synthetic peptide sequences were synthesized and tested for inhibition of CK2 activity by a non-radiometric assay of Gratz et al.^[4] Peptide B2 was identified as the

most potent CK2 inhibitor with an IC₅₀ value of 0.8 μM. It was found to be neither competitive towards ATP nor competitive towards the substrate peptide.

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P139

Auto-antibodies to αS1-Casein are Induced by Breast-Feeding

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During nursing, the immune system of newborns is challenged with multiple milk-derived proteins. Amongst them, casein proteins are main components.^[1] In particular, human αS1-casein (CSN1S1) was recently shown to possess immune-modulatory properties.^[2] We were thus interested to determine, via ELISA, if auto-antibodies to CSN1S1 are induced by breast feeding and may be sustained into adulthood.

CSN1S1 was expressed on the surface of *E. coli* using autodisplay, an efficient surface display system.^[3] ELISA plates were coated with CSN1S1-displaying bacteria, instead of the purified CSN1S1. 62 sera of healthy adult individuals who were ($n=37$) or were not ($n=25$) breast fed were investigated by the described surface display (SD)-ELISA on their IgG and IgM reaction against CSN1S1.

For cross-checking of general antibody levels, these sera were tested for anti Epstein-Barr virus (EBV) antibodies by a commercially available ELISA. To exclude cross reactivity, they were additionally tested for antibodies against bovine CSN1S1 by a homolog SD-ELISA.

Our results indicate that human CSN1S1 is an auto-antigen. There was no significant difference in antibody reaction against EBV in comparison of breast-fed and not breast-fed individuals. As well, there was no cross reaction against the bovine CSN1S1 protein in this ELISA. This underlines that CSN1S1 is the first orally determined auto-antigen, caused by breast feeding and sustaining into adult-

hood. In addition, autodisplay again proved to be a simple and rapid method to deliver antigens of human and non-human origin for a SD-ELISA.^[4]

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Surface Display of Human Hyaluronidase PH-20 and Testing of Inhibitors

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Hyaluronic acid (HA) is a linear polymer comprised of repetitive glucuronic acid and *N*-acetyl-glucosamine disaccharide units with a molecular mass up to 20 MDa. Numerous studies suggest that the balance of HA-synthesis by HA-synthases and HA-degradation by hyaluronidases has an influence on various cellular processes such as cell differentiation and proliferation, formation and progression of arthritic diseases, multiple sclerosis, wound repair and tissue hydration.^[1] Concerning their role in biological functions, hyaluronidases, such as hPH-20, are interesting targets for the development of new inhibitors as potential therapeutics for cancers and noncancer related diseases.

Numerous efforts to obtain functional hyaluronidases in *Escherichia coli* were unsuccessful due to the formation of inclusion bodies in prokaryotic expression systems. Also, expression in eukaryotic cells takes comparatively long time and yields only low amounts of protein.^[2,3] Here, we present a stains-all-based whole-cell assay for inhibitor testing of hPH-20.^[4] This whole-cell assay is established on the basis of the autodisplay technology, a surface expression system based on a secretion mechanism of Gram negative bacteria.^[5] By applying this technology, the formation of inclusion bodies is eliminated and catalytically active hPH-20 can be expressed on the surface of *E. coli* at low expenses within a short time.

Several compounds were tested for their inhibitory activity towards purified ovine testicular hyaluronidase (OTH) and towards surface displayed hPH-20. We found considerable differences in the inhibition of ovine and human hyaluronidase, showing that the results obtained with OTH are not trustworthy enough for a prediction of the inhibitory activity towards the human enzyme. By

utilizing the tool of autodisplay, hPH-20 is readily available for the testing of inhibitors, leading to the first validated inhibitors of human hyaluronidase.

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P141

Design, Synthesis and Biological Evaluation of New Diselenide Compounds as Antileishmanial Agents

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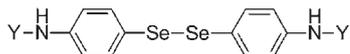
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Leishmaniasis are a spectrum of diseases caused by the protozoan *Leishmania* within the Trypanosomatidae family. They are widely spread with 12 million people affected and 350 million considered at risk with a high prevalence in undeveloped countries.^[1] Unfortunately several drugs for leishmaniasis are limited by their toxicity, development of drug chemoresistance and high cost.

Recently the trace element selenium has been identified as a new defense strategy against *Leishmania* infection. Different studies revealed that selenium interferences in the parasite's redox equilibrium through its activity on the selenocysteine group located within *Leishmania* selenoproteins.^[2] In particular, selenoprotein P is protective against oxidative damage, and it is used as protection against illness caused by *Trypanosoma*.^[3] Furthermore, in the parasites, the glutathione system is replaced by a trypanothione system, in which the reduction of sulfur groups, analogous of selenium, is carried out.

In order to continue and complete the investigation previously done by our group in selenium derivatives,^[4,5] we report the synthesis and biological evaluation of novel diselenide compounds according to the general structure shown, in which specific groups that modify both volume and polarity are introduced without changing molecular symmetry.



The antileishmanial potential of novel selenocompounds was tested in vitro against *L. infantum* amastigotes, and their cytotoxic activity was assayed against Jurkat and THP-1 cell lines to establish the selectivity index. Edelfosine and Miltefosine were used as reference drugs. The leishmanicidal activity of the most effective compounds was also tested in infected macrophages. Our results provide evidence for the potent antileishmanial activities of diselenide compounds.

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Discovery of New Small-Molecule Caspase-7 Activators

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Prenylated flavonoids have been described as a promising group of compounds with potential antitumor activity; in particular, baicalein^[1,2] and 3,7-dihydroxyflavone^[3] have been shown to induce caspase-dependent apoptosis in several human tumor cell lines. Interestingly, studies carried out by our group have demonstrated that the introduction of prenyl side chains on the scaffolds of these molecules is associated with an increase in their cell growth inhibitory effect.^[4] All these studies support the hypothesis that prenylflavonoids could represent a promising group of anticancer compounds that act by activating caspases and, therefore, by increasing cellular apoptosis.

In the present work, the modulatory effect of three prenylated flavonoids (one natural and two synthetic from CEQUIMED-UP) on the activities of caspase-3 and caspase-7 was investigated using yeast phenotypic assays, which are based on the heterologous expression of human caspase-3 or caspase-7. The activity of the compounds selected in the yeast target-based assay was also validated in human tumor cell lines that express either caspases-3 and -7 (NCI-H460) or caspase-7 but not caspase-3 (MCF-7). The levels of caspase-7 were analyzed in these cells following treatment with the compounds by western blot, and caspase activation was assessed in MCF-7 cells using the Caspase-Glo 3/7 assay. In addition, the effect of the compounds on the sensitivity of MCF-7 cells to etoposide was studied, using a cell growth inhibitory assay (sulforhodamine B assay).

The yeast target-based phenotypic assay has allowed us to identify three prenylated flavonoids as caspase-7 activators, with a higher potency than the commercially available pro-caspase-activating compound-1 (PAC-1) which is considered to be the standard activator of caspases-3 and -7. Moreover, treatment of the MCF-7 and NCI-H460 cells with these prenylated derivatives caused cellular caspase-7 cleavage in a concentration-dependent manner. Furthermore, these compounds were also found to increase the activity of caspase-7

in the MCF-7 cell line, and two of them elicited an increase in the sensitivity of MCF-7 cells to the effects of etoposide. The results obtained indicate that these prenylated flavonoids may prime tumor cells for the effect of some cytotoxic drugs.

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Lipid Rafts Behavior in CNS Disorders: A Computational Case Study for Parkinson's Disease

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Lipid rafts are highly ordered assemblies that result from the lateral segregation of certain membrane components.^[1] Studies of their peculiar composition have shown that membranes are not just a matrix where proteins reside, but are actually involved in key biological events.^[2] Recent experimental studies have demonstrated that the composition of lipid rafts is altered in some CNS disorders such as Parkinson's and Alzheimer's disease.^[3,4]

In the present study, we intend to capture the biophysical properties of lipid rafts and the impact of different membrane compositions on the aforementioned CNS diseases by applying all-atom molecular dynamics simulations. For this purpose, we simulate complex heterogeneous membrane systems consisting of different proportions of cholesterol and phospholipids, on the basis of previous experimental evidence. Characterizing various biophysical parameters such as area per lipid, membrane thickness, lipid chain order, or pair distribution functions, we are able to clearly distinguish between control and diseased membrane models. Diseased models are characterized by thicker, more condensed and more ordered membranes, whereas control models show a less restricted environment.

In summary, the current work indicates that raft compositions as found in Parkinson's or Alzheimer's disease are significantly different in their biophysical membrane properties. This has important implications for membrane-embedded signaling proteins (e.g. G-protein-coupled receptors), as their functions are tightly linked to their membrane environment.

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Study of the Biomolecular Structure in Water by Molecular Simulation

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Alcohols are amphiphilic molecules capable of hydrogen bonding. The amphiphilic character of alcohols as solutes has been observed to affect both the structure of the surrounding water and to promote their aggregation in aqueous solution. As prototype hydrogen-bonding molecules, water and alcohols (ethanol and propanol) both hold special status. Molecular dynamics (MD) and Monte Carlo (MC) simulations have been used to obtain information about the behavior of molecular liquids.

The physical properties of an aqueous solution of alcohol are characterized by nonlinear concentration dependence, first of all in the environment of low concentrations. Experimental studies^[1–4] have shown the presence of anomalous thermodynamic properties of aqueous alcohol solutions in the dependence on temperature and concentrations of the introduced substance. An X-ray diffraction experiment^[4] showed the presence of heterogeneous structures in the environments of the anomalous behavior of the thermodynamic parameters of liquid. However, neutron scattering experiments do not allow an accurate determination, at the atomic level, of which interactions lead to the appearance of these anomalies. The study of the hydration processes in solution is related to studies of structure, macroscopic behavior, and thermodynamic properties of solutions. Therefore, it is important to investigate at the molecular level and to determine the dependence between structure and the thermodynamic properties of aqueous solutions of alcohols at different fractions of alcohol in water by using Monte Carlo simulations.

Monte Carlo was performed on aqueous solutions of ethanol at various concentrations for determining the concentration regions where the local structure of the solution occurs. From the analysis of

interaction energies, radial distribution functions, and the numbers of nearest neighbors, it was found that at concentrations lower than 0.04, alcohol molecules do not influence the properties of water in water–alcohol solution. The concentration regions at which alcohol micelles are formed were determined: the role of fluctuations in forming clusters of alcohol molecules at concentration 0.18–0.22 and rebuilding ethanol clusters to micelles from ethanol molecules in the concentration range 0.30–0.38.

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P145

Discovery of a Novel Class of Antiproliferative Pyrrolo[3,2-f]quinolin-9-ones Characterized by Interfering with Both PI3K–Akt–mTOR Signaling and Microtubule Assembly

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We recently discovered a novel class of antiproliferative agents, structurally related to the pyrrolo[3,2-f]quinolin-9-one scaffold and characterized by interference with both PI3K–Akt–mTOR signaling and microtubule assembly. These represent a promising class of novel dual-target therapeutic agents. In fact, deregulation of the phosphatidylinositol 3-kinase (PI3K)–Akt–mammalian target of rapamycin (mTOR) pathway plays a central role in tumor formation and progression, providing validated targets for cancer therapy.^[1]

The newly synthesized MG-2603, selected as lead compound, showed potent antiproliferative activity in a panel of human tumor cell lines, especially in leukemic cells. From a mechanistic point of view, MG-2603 possesses inhibitory activity against both PI3K and mTOR; it caused a significant concentration-dependent decrease in phosphorylation (at Ser473) of the Akt protein, whereas total Akt protein expression remained unaltered. Moreover, it also decreased the phosphorylation of mTOR and its downstream targets, p70 ribosomal S6 kinase and 4E-BP1. Effects of these compounds on the PI3K–Akt–mTOR pathway were determined by western blot analysis.

Further study revealed that MG-2603 inhibits tubulin polymerization by binding to the colchicine binding site of tubulin, resulting in microtubule disturbance. The interactions between MG-2603 and tubulin were investigated by polymerization assay and inhibition of colchicine binding. Its potency for inhibition of tubulin polymerization is similar to that of the reference compound, combretastatin-A4. Molecular modeling indicates that MG-2603 could bind to the kinase domains of the PI3K p110a subunit and mTOR, and that MG-2603 shares similar hydrophobic interactions with colchicines in complex with tubulin.

In addition, MG-2603 induced rapid apoptosis in tumor cells, which might reflect a synergistic cooperation between blockade of both PI3–Akt–mTOR signaling and the tubulin cytoskeleton. In conclusion, targeting both PI3K–Akt–mTOR signaling and cytoskeleton microtubules contributes to the antitumor activity of MG-2603 and provides new clues for anticancer drug design and development.

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P147

Ligand- and Structure-Based Modeling of Human Aryl Sulfotransferase 1A1 Activity

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Sulfonation catalyzed by sulfotransferases plays an important role in chemical defense mechanisms against various xenobiotics. A major human sulfotransferase, SUL1A1, metabolizes and/or bioactivates many endogenous compounds and is implicated in a range of cancers because of its ability to modify diverse pro-mutagen and pro-carcinogen xenobiotics.

We examined the binding patterns of various substrates to SUL1A1 by using LigandScout^[1] through a combination of ligand- and protein-based modeling approaches. First, we developed and validated a structure-based pharmacophore model for SUL1A1, resulting in a model with high specificity excluding all inactive molecules. Second, we constructed and validated a ligand-based pharmacophore model for 1A1 substrates using more than 70 substrates covering several activity classes and different chemical scaffolds. Our study provides insight into the molecular mechanisms of interaction of various substrates with human SUL1A1.

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Development of a Whole-Cell Assay for the Determination of Human Protein Kinase CK2 Activity and Testing of Inhibitors

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Human CK2 is a constitutively active protein kinase which is known to phosphorylate a vast number of substrates.^[1] Its participation in many different kinds of human diseases, particularly malignant cell transformations, has been extensively reported.^[2] Furthermore, there is growing evidence that down-regulation of CK2 activity in tumor cells causes apoptosis and therefore decreases cell viability, suggesting CK2 inhibitors as potential therapeutics for the treatment of cancer.^[3] Thus, the identification and evaluation of compounds with inhibitory activity toward human protein kinase CK2 is an important step in the early stage of the discovery of potential tumor therapeutics.

Access to human target enzymes is often a limiting factor for inhibitor testing procedures. Hence, the expression of the desired enzyme at the cell surface of *Escherichia coli* via Autodisplay^[4] is a promising technique to circumvent this issue, and offers additional advantages such as stabilized enzymes through membrane attachment and the possibility to use the surface-presented enzymes more than once. Moreover, the Autodisplay system allows identification of novel inhibitors of human enzymes, as already demonstrated for the human hyaluronidase hPH20.^[5] Here we present a novel CE-based assay^[6] for activity determination of human protein kinase CK2 displayed at the cell surface of *E. coli*. With this assay we quantified the inhibitory potency of the well-studied CK2 inhibitor Emodin. Our results are in good accordance with reported data,^[7] showing the applicability of this assay for the purpose of drug discovery. TBB as another known CK2 inhibitor will be evaluated for comparison, and novel compounds as potential CK2 inhibitors will be investigated.

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P149

In the Search of Electrostatic Complementarity in GPCR Dimer Interfaces

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Electrostatic forces are key regulators in a variety of biological processes. They play a fundamental role in protein structure, function, recognition, and association,^[1] as well as contribute to protein dynamic properties.^[2]

In the present work, we focus our attention on G-protein-coupled receptor (GPCR) dimers, which are gaining increased acceptance as drug targets for diverse diseases. Here we report a novel protocol for electrostatically characterizing the GPCR interfaces on different dimers. The protocol uses APBS software for solving the Poisson–Boltzmann equation. Importantly, the protocol takes into account: 1) the correct protonation states of each protomer (PROPKA algorithm), 2) the lipid membrane environment in the transmembrane helical receptor bundle, and 3) aqueous solvation in both the extracellular and intracellular regions of the GPCR dimer. Finally, we apply an in-house-developed algorithm for assessing the biophysical representativeness of the GPCR complex on the basis of the electrostatic complementarity of the dimer interface.

This protocol was validated by comparing the electrostatic properties of numerous dimer constructs with the corresponding X-ray structure. The results indicate that electrostatic complementarity is favored in the true dimer structure. All in all, the outcome of this study suggests that electrostatics could be a valid parameter for identifying physiologically relevant GPCR dimers which can be used as targets for developing novel therapeutic agents.

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P150

Discovery of 4,5'-Bithiazoles as Novel Inhibitors of DNA Gyrase B by a Structure-Based Virtual Screening Approach

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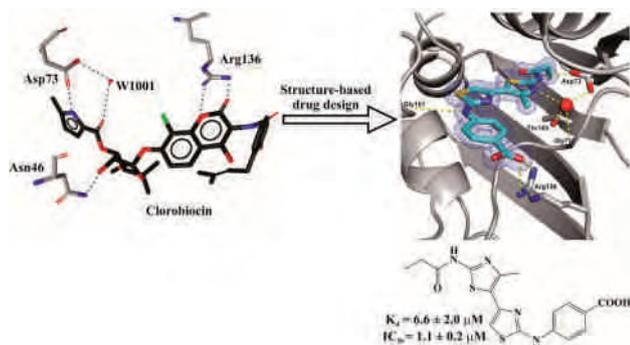
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The wide use of antibiotics in previous decades has resulted in the increased incidence of bacterial resistance to most of the available antibacterials and is driving an urgent need for the development of novel and effective antibacterial agents.^[1] The main challenge remains the discovery of highly potent antibacterials with a broad spectrum of efficacy and improved safety profile.^[2] One of the well-established and validated targets of antibacterial drug design is DNA gyrase,^[3,4] a unique bacterial type II topoisomerase originating from the superfamily of gyrase, Hsp90, histidine kinase, and MutL (GHKL) enzymes; it catalyzes the introduction of negative supercoils into the DNA molecule using concurrent ATP hydrolysis.



Coumarins^[5,6] are a class of bacterial DNA gyrase B (GyrB) subunit inhibitors that target its ATP binding site. Starting from the available information about the clorobiocin binding mode,^[7] a two-step in silico virtual screening campaign was designed, combining molecular docking calculations with three-dimensional structure-based pharmacophore information. A novel class of 4'-methyl-N²-phenyl-[4,5'-bithiazole]-2,2'-diamine inhibitors with low micromolar antigyrase activity and moderate in vivo antibacterial activity was discovered and subsequently characterized using several different biophysical techniques, such as surface plasmon resonance (SPR), microscale thermophoresis (MST), and differential scanning fluorimetry (DSF). The binding mode of the most potent compound predicted by our model was further successfully confirmed by X-ray crystallography.

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Development of Novel Highly Potent Substrate Analogue Inhibitors of Furin

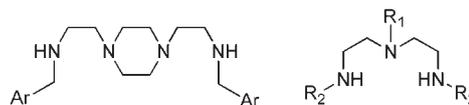
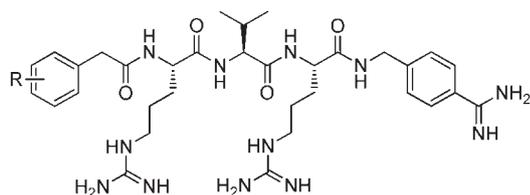
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The type-I transmembrane protein furin belongs to the family of proprotein convertases (PCs) and contains a Ca²⁺-dependent subtilisin-like serine protease domain. Furin is ubiquitously distributed in human tissues and catalyzes the maturation of precursor proteins. In addition to its normal physiological role, furin also contributes to the activation of many disease-related proteins. It is involved in various viral and bacterial infections, tumorigenesis, neurodegenerative disorders, diabetes, and atherosclerosis. Therefore, furin has emerged as a potential target for drug design.

Furin has a strong preference for processing its substrates at a multibasic cleavage site, like Arg-X-Arg/Lys-Arg↓-X. Derived from this sequence we recently developed initial substrate analogue inhibitors containing decarboxylated arginine mimetics at the P1 position.^[1,2] The most potent analogues of this first series inhibit furin with K_i values of ~1 nM. Further substitution of the N-terminal phenylacetyl group with basic residues in a new series has significantly improved the affinity, to inhibition constants <20 pM. Selectivity studies with related furin-like PCs revealed a similar potency in the picomolar range against PC1/3, PC4, PC5/6, and PACE4, whereas these inhibitors possess poor affinity against PC2 and PC7 or various trypsin-like serine proteases.

Selected derivatives were effective in the inhibition of hemagglutinin cleavage and propagation of highly pathogenic avian H5N1 and H7N1 influenza virus strains and decreased Shiga toxin activation in HEp-2 cells. This antiviral effect along with the protective effect against a bacterial toxin both suggest that inhibitors of furin or furin-like proprotein convertases could represent promising lead structures for future drug development, in particular for the treatment of infectious diseases. The inhibition of host cell proteases such as furin could be a promising approach to avoid the often-observed emergence of resistance due to mutations in the commonly addressed viral targets.



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P152

Synthesis of New Polyamine–Polyamide Ligands: Evaluation as Antiparasitic Drugs

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Tropical diseases caused by parasitic pathogens are among the most significant causes for the high mortality rates in developing countries. Leishmaniasis is one of the 14 neglected diseases on the Tropical Diseases Research (TDR) list of the World Health Organization (WHO).^[1]

The biological polyamines—putrescine, spermidine, and spermine—occur in higher concentrations in cells with increased proliferation rates such as parasitic pathogens. Polyamines are involved in a variety of important functions and are essential for cell growth, differentiation, and proliferation.^[2] Trypanothione, a spermidine-bis(glutathionyl) conjugate, is essential to *Trypanosoma* and *Leishmania* because it is involved in the parasites' defense against oxidative stress. Serious damage is occurs in the parasite cycle if trypanothione cannot be formed due to decreasing spermidine concentrations.^[3] In this regard, it has been shown that alkyl-aryl-substituted polyamines may interfere with the functioning of biological polyamines.^[4,5]

In this study, we investigated the leishmanicidal effect of a series of polyamine derivatives (shown) against promastigote forms of *Leishmania* spp. Cytotoxic properties were evaluated against J774 macrophages. The discovery of some polyamines as potent anti-leishmanial compounds with high selectivity is presented along with their SAR analysis.

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Computational De Novo Design of Peptide Ligands

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Therapeutic peptides are attracting increasing interest in medicinal chemistry and the pharmaceutical industry. Synthetic peptides offer a range of advantages over small-molecule drugs, antibodies, and recombinant proteins. They are less immunogenic, more stable, have decreased potential for interaction with the immune system, better organ and tumor penetration, and lower manufacturing costs.^[1] Unlike small molecules, peptides are also able to target large protein-protein interfaces with high selectivity and specificity.

Although molecular modeling of small molecules is an integral component of modern drug discovery, the higher flexibility of peptides has so far hampered the implementation of de novo design algorithms for peptides. Addressing this limitation, we have developed a new algorithm for the de novo design of peptide ligands for binding pockets of protein targets. To evaluate this algorithm, we used it to design, generate, and evaluate peptide ligands for the NGF binding site of TrkA.

TrkA belongs to the neurotrophic tyrosine kinase receptor type I family. It is a single transmembrane protein and a target for various neurotrophins, including the brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), neurotrophin 4/5, as well as nerve growth factor (NGF), which has the highest affinity (10^{-9} M) to the receptor (Figure 1a).^[2] Because the TrkA-NGF interaction is involved in the transmission of chronic pain, inhibitors of this interaction are candidates for novel strategies in pain therapy.

Therefore, we synthesized a range of peptides proposed by our new algorithm, and tested them for binding to NGF, as well as for inhibition of signal transduction initiated by the TrkA–NGF interaction (Figure 1b).

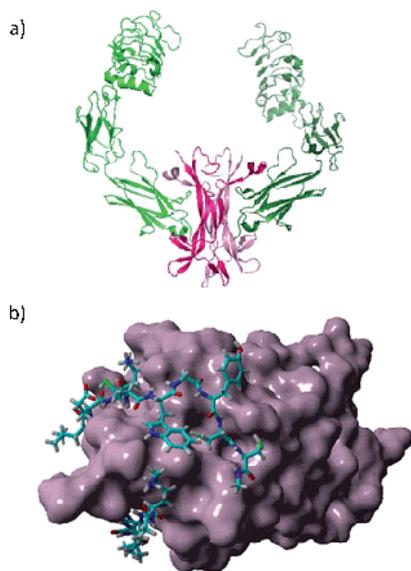


Figure 1. a) Crystal structure of homodimeric human TrkA in complex with homodimeric human NGF (PDB: 1WWW); b) Molecular surface of domain 5 of TrkA in complex with a calculated peptide.

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P154

In Silico Screening of Inhibitors for Sumoylation Enzyme Ubc9

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Sumoylation is a post-translational modification that plays an important role in a wide range of cellular processes including DNA replication and repair, chromosome packing and dynamics, genome integrity, nuclear transport, signal transduction, and cell proliferation. Among the proteins involved in the sumoylation pathway, Ubc9 is the sole E2-conjugating enzyme required for sumoylation and plays a central role by interacting with almost all the partners required for sumoylation. Ubc9 has been implicated in a variety of human malignancies such as ovarian carcinoma, melanoma, and lung adenocarcinoma, suggesting that Ubc9 inhibition could be a potential therapeutic approach to control tumorigenesis. To exploit

the therapeutic potential of Ubc9, we used an in silico approach to find the possible binding site of a known inhibitor, spectomycin B1, by using molecular docking and molecular dynamics simulations. The structural information derived was then used to identify potential small molecules that target Ubc9 using a hybrid structure-based virtual screening protocol that incorporates both ligand- and structure-based techniques.

The virtual screening procedure employed is a combination of rapid three-dimensional ligand-shape-based screening with two stages of flexible docking followed by prioritization of potent compounds using molecular dynamics simulation and binding free energy calculations to select compounds for biological testing. Initially, the group of lead-like compounds from the ZINC database was decreased by removing compounds distant from spectomycin B1 in terms of three-dimensional structure. The resulting hits were then subjected to full flexible molecular docking simulations to select compounds on the basis of their ability to form favorable interactions with the inhibitor binding site. Finally, selection of potent compounds was done based on molecular dynamics simulations and binding free energy profiles. There are seven compounds that showed micromolar activity in the in vitro sumoylation assay out of 19 compounds identified by virtual screening. Further chemical optimization of these inhibitors is underway.

P155

Aziridine-Based Inhibitors of HIV-1 Protease

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HIV-1 protease (PR) is an aspartyl protease essential for proper virion assembly and maturation. Many competitive inhibitors of this protease are available with FDA approval, but there is a rapid rise of strains that encode mutant proteases resistant to these reversible protease inhibitors.^[1] We explored the ability of QM/MM models to accurately describe the inhibition reaction of HIV-1 PR by epoxide- and aziridine-based inhibitors. In contrast to their epoxide counterparts, the mechanisms and binding modes of aziridine-based inhibitors have been the subject much less investigation; for example no X-ray measurements for complexes with HIV-1 PR or SIV PR are available. Computations predict their inhibition mechanism to be similar to that of epoxides, but differences result from the stronger basicity of aziridine.^[2] Accordingly, aziridine-based inhibitors should be ideally suited for aspartyl proteases, which act in more acidic environments.^[3] This was indeed shown by recent work. By employing docking approaches, the HIV PR structure is used to predict possible substitution patterns of such new inhibitors with improved binding affinities. We present synthetic approaches to these new optimized aziridine-based inhibitors.

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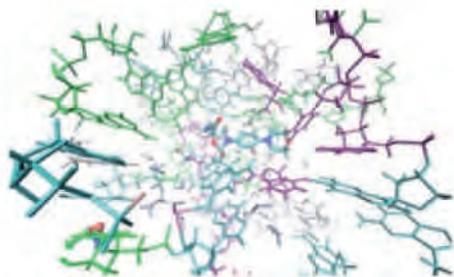
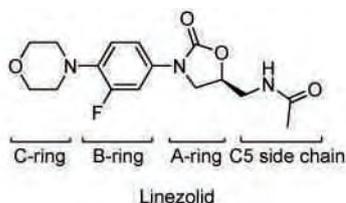
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P156

Modeling of Drug–Ribosome Interactions in the Design of Linezolid-Like Compounds

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Linezolid belongs to the newly developed class of oxazolidinone antibiotics.^[1] Its mechanism of action involves binding with the 50S ribosomal subunit to prevent the formation of a functional initiation complex 70S for the synthesis of proteins.^[2,3] Because resistance toward Linezolid has recently emerged, researchers have become interested in the design of new Linezolid-like molecules to counteract this phenomenon.

As preliminary results of a research project on the molecular design of heterocycle-based antibacterials to combat multidrug resistance (MDR),^[4,5] we report the results concerning the modeling of

drug–ribosome interactions to evaluate the affinity of new Linezolid-like compounds for their biological target. Synthesis and activity data of selected compounds are also presented.

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P157

New Polyamine–Polyamide Ligands as Anticancer Drugs

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Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The increased incidence of cancer in developing countries is a result of aging populations and cancer-associated lifestyles such as smoking, alcohol consumption, physical inactivity and western diets.^[1]

Polyamines are essential biological compounds in eukaryotes, participating in a variety of important functions such as cell growth, proliferation, and differentiation. The biological concentrations of polyamines (putrescine, spermidine, and spermine) increase in cells that display elevated proliferation rates such as cancer cells. In biological systems, natural polyamines bind to polyanions and to proteins with anionic sites. Several derivatives and analogues of biological polyamines were recently synthesized to generate a new type of anticancer drug.^[2]

Herein we describe the synthesis of several polyamine-amide compounds. The compounds were evaluated for their in vitro antiproliferative activities against a panel of eleven human cancer cell lines, including glioblastoma, colorectal cancer, non-Hodgkin lymphoma, and acute T-cell leukemia. Cell viability was determined by quantification of ATP, which signals the presence of metabolically active cells, by using the Cell Titer-Glo Luminiscent assay. The indicated human cancer cell lines were plated in 96-well plates one day before treating them with vehicle alone as a control or with the indicated compounds at 10 μM ; 48 h after treatment, cell viability was monitored using the Cell Titer-Glo reagent. Luminiscent was detected with a multi-well scanning spectrophotometer. Cell viability is represented as a percentage relative to vehicle-treated cells. In this communication, we report the synthesis and details of the biological data.

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P158

Synthesis of Novel Semicarbazide-Sensitive Amine Oxidase Inhibitors via *tert*-Amino Effect

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Semicarbazide-sensitive amine oxidases (SSAOs) [EC 1.4.3.6.] belong to the family of copper-containing amine oxidases. SSAO has a dual function: 1) it catalyzes the oxidative deamination of primary aliphatic and aromatic amines with formation of the corresponding aldehyde, H_2O_2 , and NH_3 , with possible formation of cytotoxic products; 2) being identical with vascular adhesion protein-1 (VAP-1), it has a role in the adhesion of lymphocytes to endothelial cells. There is growing evidence for the involvement of SSAO in inflammation; its potential role as a therapeutic target for inhibitors is currently under investigation. Moreover, the substrates of SSAO are also of interest, as they possess insulin-like properties.^[1]

Our goal is to develop novel inhibitors and substrates of SSAO/VAP-1 for the treatment of various inflammatory conditions and for possible treatment of diabetes, respectively. We recently discovered a novel class of inhibitor.^[2] As a continuation of these studies, we report a novel series of fused tetrahydroquinolines.

One version of the type II *tert*-amino effect operates with *tert*-anilines possessing an *ortho*-vinyl substituent to afford tetrahydroquinolines by a thermal isomerization process;^[3] the reaction could also be extended to heteroaryl and biaryl systems.^[4,5] The formation of tetrahydropyrido-fused ring systems via the *tert*-amino effect involves a three-step, convenient, rapid, and eco-friendly microwave-assisted one-pot process, starting from commercially available *ortho*-fluoro aromatic aldehydes or ketones.^[6] Further transformation of the cyclized products led to some novel compounds influencing SSAO.

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P159

Autodisplay of the Human Chaperone Hsp90 and Development of an Inhibitor Assay

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Hsp90 is a ubiquitous molecular chaperone and makes up 1–2% of soluble cell protein in cells under normal conditions.^[1] It assists in the folding and activation of numerous essential proteins including kinases, polymerases, and transcription factors.^[2] Some of these proteins are involved in tumor formation and growth which makes Hsp90 an interesting drug target for cancer treatment. Although there have been numerous inhibitors discovered and studied over the last years, there is no small molecule that inhibits the dimerization of Hsp90. A new computational strategy aims to identify the protein–protein interaction site and the requirements for small molecules to binding this site with high affinity.^[3]

The expression of human enzymes on the cell surface of *Escherichia coli* through Autodisplay is a strong tool for the development of assays for human enzymes, which can be used to test inhibitors.^[4] Here we present the cell-surface display of the human chaperone Hsp90 in an active and dimeric form. We show strong evidence for the dimerization of Hsp90, including outer membrane isolation, SDS PAGE, and western blot analysis. A FACS analysis confirmed the binding of FITC-labeled p53 to autodisplayed Hsp90.

Through computational analysis, hot-spot prediction of the dimerization site of Hsp90 was made. The result of this prediction suggests that several residues at the C terminus of Hsp90 are responsible for dimerization. We intend to mutate the suggested amino acid residues, thus preventing dimerization of Hsp90. In the event this proof of principle is successful, studies of FITC-labeled p53 binding to autodisplayed non-modified Hsp90 can be used to develop new compounds for the inhibition of dimerization, which could result in a new approach in tumor therapy: inhibition of protein–protein interaction by small-molecule drugs.

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P160

Allosteric Modulation of GPCRs by the Membrane Environment

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The function of G-protein-coupled receptors (GPCRs) is very sensitive to the membrane environment. There is clear evidence that the lipid composition of membranes plays a crucial role in the modulation of transmembrane proteins.^[1,2] For GPCRs, it is unclear if this effect is primarily mediated by specific lipid–receptor interactions or rather by an unspecific effect based on the alteration of membrane properties (e.g., thickness). Understanding this well-recognized but often neglected connection between GPCR function and membrane properties at the molecular level can help to unveil new allosteric regulatory sites, leading to new drug discovery strategies.

For this purpose, we simulate GPCRs in multi-component membrane systems using molecular dynamics at the microsecond timescale. On the one hand, we address the complex nature of heterogeneous membranes by simulating different lipid types and proportions.^[3] The biophysical properties of these heterogeneous membrane models are in good agreement with experimental values. On the other hand, by embedding GPCRs in such a realistic membrane environment, we are able to detect specific lipid–receptor interactions which may represent putative allosteric regulatory sites. Moreover, our results indicate that unspecific membrane effects, mainly mediated by membrane thickness, also play a crucial role in receptor conformation.^[4]

All in all, the present study stresses that 1) conformational states of GPCRs are tightly linked to membrane composition and 2) modeling membrane effects can be a useful approach to detect allosteric regulatory sites.

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P161

In Vitro Studies of the Wound-Healing Properties of *Chelidonium majus* Flower Extracts

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Chelidonium majus (Greater Celandine) is a perennial grass, ~40–80 cm tall, that belongs to the family of *Papaveraceae*. In Italy, it may be found in both Mediterranean and mountainous regions, where it grows spontaneously in uncultivated fields, stones and walls.^[1] The Celandine has a thin and slightly hairy stalk, highly branched and with turgid knots. Leaves are alternate, lobate, odd-pinnate, finely hairy, with a bluish–green color on the top face and greyish on the bottom. Flowers are made by a cross-shaped corolla of four yellow petal with numerous central stamina. The fruit is a green silique containing a row of small light-yellow seeds. Roots are brownish–orange taproot, and it is easy to distinguish the primary root from the secondary ones. The most remarkable trait of the plant is the

presence of orange latex, which spurts out when the branch is cut and which oxidizes rapidly, turning into a brownish–black color upon contact with air.^[2,3]

It is a well-known plant in folk medicine, where it is known as “Warts’ grass” because of its use in the treatment of warts, calluses, and corns. In particular, in Sardinia, it is used as an ingredient of ethnobotanic preparations with wound-healing effects.

In this study, in vitro wound-healing assays were carried out on several *C. majus* extracts to investigate their modulatory activity on both proliferation and migration of human dermal fibroblasts, epidermal keratinocytes, and human umbilical vein endothelial cells. Interestingly, at various concentrations, *C. majus* flower extracts were able to stimulate proliferation and migration of fibroblasts, keratinocytes, and endothelial cells.

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P162

Microwave-Assisted Synthesis and Biological Properties of Some New Pyrazinamide Derivatives

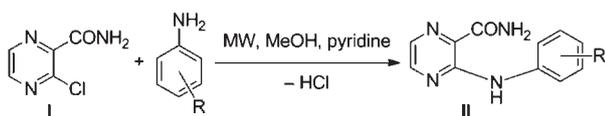
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The number of new tuberculosis (TB) cases has fallen slowly over the past few years. However, new obstacles have emerged, with mycobacterial strains that have become increasingly resistant to current treatments, and with HIV co-infections. It has turned into an epidemiological problem, and the World Health Organization (WHO) has been focusing on it from the beginning.^[1]

The small molecule pyrazinamide, the first-line anti-TB drug, is very suitable for chemical modification and is a model for substances prepared in this research project. 3-Chloropyrazine-2-carboxamide (I) as a starting compound, treated with a group of various aromatic amines (using a microwave reactor with focused field), yielded N-substituted 3-aminopyrazine-2-carboxamides II.



The prepared structures were characterized by melting point, IR and NMR spectra, log *P* and elemental analysis. In vitro biological screens were carried out as the next step. These involved anti-mycobacterial screens (various *Mycobacterium* species; pyrazinamide and isoniazide as standards), antibacterial and antifungal screens (eight bacterial and eight fungal stems; neomycin, bacitracin, penicillin G, ciprofloxacin, phenoxymethylpenicillin, amphotericin B, voriconazole, nystatin, and fluconazole as standards), and testing for herbicidal activity (inhibition of photosynthetic electron transport in spinach chloroplasts with DCMU (Diuron) as a standard; IC₅₀). Six of the prepared compounds showed some herbicidal activity, but not with activity as good as the standard (IC₅₀ for DCMU: 1.9 μM).

Acknowledgements: This study was supported by the Grant Agency of Charles University (B-CH/710312), by Ministry of Health of Czech Republic (IGA NZ 13346) and by Ministry of Education, Youth and Sports of Czech Republic (SVV-2012-265-001).

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P163

Designing Substrate-Mimicking Compounds as Sirtuin Modulators

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Sirtuins are a family of NAD⁺-dependent histone deacetylases/ADP ribosyltransferases that are involved in several biological processes such as aging, DNA repair, and metabolic regulation. Sirtuins are evolutionarily conserved across mammals. Of all mammalian sirtuins, SIRT1, SIRT2, and SIRT3 exhibit the most robust deacetylase activity on a variety of natural and synthetic acetylated substrates. Deacetylase activity has also been detected for SIRT6.

Several substrate-based peptides have been reported to inhibit sirtuins. The peptidic inhibitors can have various sequences which reflect the fact that sirtuins can deacetylate various substrates. Although peptidic compounds may show advantages over small molecules in terms of specificity and affinity for different targets, they do not possess drug-like properties. We recently designed a series of substrate-mimicking compounds that overcome the problems with peptides.^[1]

We synthesized the thioacetyllysine residue with various modifications at the C- and N-terminal sites. In the design we used the information from the hydrogen bond network between the substrate main chain and the backbone of protein residues Gly295, Glu296, Glu323, and Glu325. In addition, molecular docking was used in this study to screen chemical databases for SIRT3 inhibitors. Com-

pounds were selected for in vitro testing based on docking scores and interaction analysis. The selected compounds were tested in a fluorescence-based assay. Several new compounds with SIRT3 inhibitory activity were found in the first screening runs. The active compounds were used in the subsequent virtual screening steps to guide further compound selection.

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P164

Selectivity Profile Analysis of the CDC2-Like Kinase Inhibitor TG003 by Docking with GOLD

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CDC2-like kinases (CLKs) affect protein expression by phosphorylation of splicing factors. Various diseases such as Alzheimer's disease are influenced by changes in gene regulation. Hence, CDC2-like kinases could prove to be a valuable drug target.^[1] Specific inhibitors of CLKs are pivotal for acquiring more detailed information on the biological role and druggability of these kinases. However, to date few inhibitors with only modest selectivity for CLKs are known. Predicting the inhibitory properties of a compound with in silico tools prior to synthesis could lead to a more focused and efficient experimental approach. Therefore we investigated if molecular docking is applicable for a selectivity profile prediction of novel CLK inhibitors by analyzing docking results of the known CLK inhibitor TG003 for all isoforms.^[2] As there is no crystal structure of CLK4 available, we created a homology model with MODELLER using CLK1 as template.

When crystal structures were used in the docking process, differences in IC₅₀ values of TG003 over the CLK isoforms correlated with a deviation in the observed binding modes predicted by GOLD, thus rendering the described method a useful asset for the design of novel selective CLK inhibitors. To improve the predictive capabilities of the CLK4 homology model, however, further investigations are necessary.

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P165

Treatment of Psychiatric Disorders: Allosteric Action of Lithium Ions on GPCRs?

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Lithium belongs to a small family of structurally disparate drugs used for the management of psychiatric disorders such as bipolar disorders, schizophrenia, and depression.^[1] Despite its indispensable application in psychiatric therapy, the underlying molecular mechanism of lithium action in the human body is not well understood. There is a strong body of evidence that classical and atypical antipsychotic drugs exert part of their pharmacological effects through various G-protein coupled receptors (GPCRs).^[2]

To elucidate whether lithium mediates some of its multifaceted actions via GPCRs too, we carried out extended molecular dynamics simulations of the dopaminergic D₂ GPCR embedded in a realistic membrane environment, in the presence of anti-psychotically active lithium ions as well as anti-psychotically inactive sodium and potassium ions. The outcome of our study indicates that the presence of lithium ions leads to important structural changes on the dopaminergic D₂ receptor relative to the inactive sodium and potassium ions that could have dramatic effects on the function of this GPCR. In detail, we found that lithium ions act allosterically on the extracellular loop 2 (ECL2), inducing a conformational change that partially closes the receptor entrance and simultaneously affects the architecture of the orthosteric binding pocket. The partial receptor closure is enabled by the unique ability of the small-sized lithium ion to form a particular salt bridge between the ECL2 and the extracellular end of TM2 which is not observed for the larger-sized sodium and potassium ions.

All in all, this work provides new insight into an allosteric mechanism at the dopaminergic D₂ receptor by which anti-psychotically active lithium may mediate part of its beneficial action in the treatment of psychotic disorders, and proposes new drug discovery strategies for allosteric modulators.

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P166

Inhibitors of sEH Phosphatase Activity Found by Virtual, Biophysical and Biochemical Screening

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Soluble epoxide hydrolase (sEH) catalyzes the conversion of epoxyeicosatrienoic acids (EETs), lipid mediators with anti-inflammatory and cardiovascular protective properties, to dihydroxyeicosatrienoic acids (DHETs).^[1] This reaction is part of the arachidonic acid cascade. The enzyme contains two distinct domains: the well-studied C-terminal epoxide hydrolase domain and the N-terminal phosphatase domain.^[2] The latter catalyzes the hydrolysis of phosphate monoesters, isoprenoid and lipid phosphates.^[3] The biological function of this N-terminal domain is unknown so far and phosphatase activity remained unaffected by typical phosphatase inhibitors. Therefore, further development of inhibitors is required.

We present a computer-aided fragment-based approach to screen for novel sEH-phosphatase inhibitors. We performed a molecular docking study with compounds filtered from a commercially available library by applying the "Astex rule of 3".^[4] These fragment-like compounds were docked into the phosphatase binding site of the X-ray structure of sEH available from the Protein Data Bank (PDB code 1VJ5^[5]) using MOE software. The 60 top-scored ligands were further manually evaluated with regard to chemical diversity. The 30 purchased ligands were in vitro evaluated in a phosphatase fluorescence-based activity assay.^[6] Additionally the receptor–ligand interactions were confirmed by STD-NMR studies,^[7] and binding efficacy indices^[8] were calculated. The most promising candidates served as a query for a subsequent substructure search and yielded several hits. These hits were further modified by means of chemical synthesis leading to compounds that showed inhibitory activity in the low micromolar range.

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P167

From Molecular Docking to 3D Quantitative Structure–Activity Relationships (3D-QSAR): Insights into the Binding Mode of 5-Lipoxygenase Inhibitors

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Pharmacological intervention with 5-lipoxygenase (5-LO) is a promising strategy for the treatment of inflammatory and allergic ailments including asthma. With the aim of developing predictive models of 5-LO affinity and gaining insight into the molecular basis of ligand–target interactions, we describe herein QSAR studies of 59 diverse non-redox-competitive 5-LO inhibitors based on the use of molecular shape descriptors and docking experiments. These studies have successfully yielded a predictive model that is able to explain much of the variance in the activity of the training set compounds while satisfactorily predicting the 5-LO inhibitory activity of an external test set of compounds. Inspection of the selected variables in the QSAR equation unveils the importance of specific interactions which are observed from docking experiments. Collectively, these results may be used to design novel potent and selective non-redox 5-LO inhibitors.

P168

Enantioselective Synthesis of the Non-proteinogenic Amino Acid 2,4-Diamino-3,3-dimethylbutyric Acid (Ddb) and Its Use in Solid-Phase Peptide Synthesis

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Non-natural amino acids are useful molecules to investigate the structure–activity relationship of biologically active peptides and their targets. Our research group contributed to the medicinal chemistry of neuropeptide S (NPS), a 20-mer peptide able to stimulate arousal and evoke anxiolytic-like effects. Such studies identified position 5 of NPS as crucial for the activation of the NPS receptor (NPSR) and indicated that D chirality associated to a bulky aliphatic side chain is needed for high potency antagonism.^[1] We therefore decided to synthesize the non-natural amino acid Ddb and use it for the generation of [Ddb⁵]NPS. Figure 1 depicts the retrosynthetic analysis of Ddb orthogonally protected using an organocatalytic approach.

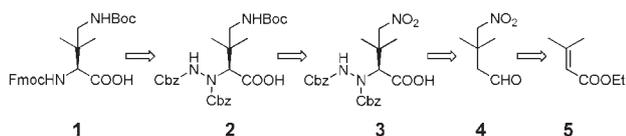


Figure 1.

The orthogonally protected amino acid **1** could be obtained from compound **2** by hydrogenation and Fmoc protection. Compound **2** could be obtained from compound **3** by reduction of the nitro group and Boc protection of the corresponding amine. Compound **3** could be obtained from aldehyde **4** by proline-catalyzed α -amination;^[2] nitroaldehyde **4** could be simply obtained by Michael addition of nitromethane with commercially available ester **5**.

The synthesis of compound **3** was achieved in good yield and in both enantiomeric forms using D-Pro and L-Pro as a catalyst in the α -amination reaction. Chiral HPLC using Lux 1 column and NMR spectra confirmed the purity of the final amino acid. D-Ddb was then used for the solid-phase synthesis of [D-Ddb⁵]NPS. Analytical HPLC and mass spectrometric analyses confirmed the purity of the desired peptide (Figure 2).

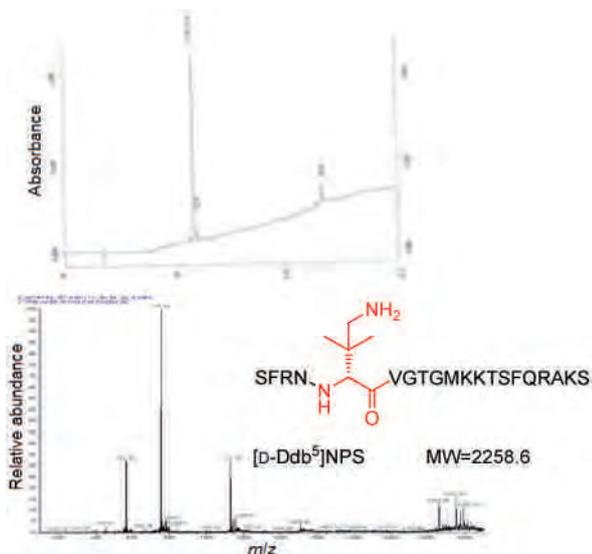


Figure 2.

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P169

Bicyclic Acetals: Potential Inhibitors of Golgi α -Mannosidase II

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Cancer—predominantly tracheal, bronchus and lung cancer—causes 5.9% (third place in the top ten after ischemic heart disease and stroke) of deaths in high-income countries and 2.4% (seventh place) of deaths in the world. Unregulated spreading of cells, invasion of healthy tissue, and especially metastasis characterize the pathology of these diseases. Selectins, carbohydrate-recognizing proteins, play a crucial role in binding metastasizing cancer cells. Ligands for selectins are often modified glycosylation patterns on the outer surface of the cancer cells. Studies have shown an overexpression of different sugar-hydrolyzing enzymes in these cells, making such enzymes promising targets for new anticancer drugs. Especially inhibition of the golgi α -mannosidase II (GM II) has shown tumor repression.^[1]

GM II, a glycosyl hydrolase, is a 125 kDa type II transmembrane protein that plays an essential role in the N-glycosylation pathway of asparagine side chains. The high specific cleavage of two mannose units [α -(1,3) and α -(1,3)] of the intermediate GlcNAcMan₅ (GlcNAc)₂ takes place in the active site of the enzyme, with two aspartate residues and a zinc cation involved.^[2] GM II is a retaining glycosidase and cleaves the sugars in a two-step S_N2 mechanism that preserves the configuration of the anomeric C atom. Currently available inhibitors, mostly derivatives of swainsonine, have various side effects due to low selectivity. The goal of the presented project is the synthesis of selective, covalent reversible inhibitors with a long resting time in the catalytic site of the enzyme. QM calculations and docking simulations have shown that bicyclic acetals are promising candidates in terms of both high affinity to the target enzyme and reaction kinetics.^[3] Based on L-gulose, we synthesized 1-2 and 1-6 bridged species. We used known strategies for the synthesis of potential inhibitors. New strategies such as cycloaddition and especially olefin ring-closing metathesis (RCM) are promising alternative ways to access the desired acetals.

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P170

LC-MS/MS-Based Quantification of Endogenous GABA in HEK293 and COS-7

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γ -Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the CNS. After the arrival of an excitation potential at the presynaptic neuron, GABA is released into the synaptic cleft where it can bind to GABA receptors and cause hyperpolarization. To terminate signal transduction, GABA must be eliminated from the synaptic cleft. This is managed by reuptake into the neurons or the glia cells via specific transporters (GAT1–4). As GAT inhibitors are able to enhance GABA neurotransmitter function in the synaptic cleft, they represent promising drug targets for several diseases such as epilepsy, Parkinson's disease, and Huntington's chorea, which are associated with imbalances in GABA neurotransmission.

Screening for new, potent, and subtype-specific GAT inhibitors is nearly exclusively based on [3 H]GABA uptake assays employing GAT-transfected eukaryotic cell cultures (HEK293, COS-7, etc.). The aim of the present study was to determine endogenous GABA levels in the respective cell lines serving as GAT source in uptake assays, as these GABA levels may influence the uptake rate, amount, and efficiency.

Therefore, we developed a simple and reliable method for the quantification of intracellular GABA levels in various cell types via LC-MS/MS. Using a YMC-PVA-Sil-HILIC column (50 \times 2.1 mm) in combination with a mobile phase consisting of 70% acetonitrile and 30% NH₄HCO₃ buffer (10 mM, pH 8.5) provided sufficient retention of GABA without derivatization. An API 5000 triple quadrupole mass spectrometer operated in the positive MRM mode enabled highly sensitive GABA detection, recording the mass transitions m/z 104 \rightarrow 87, 104 \rightarrow 86, and 104 \rightarrow 69. Intracellular concentration of endogenous GABA could be quantified via a deuterated internal standard ([2 H₂]-GABA). The developed method with an LLOQ of 625 pM GABA (solvent standard) is more sensitive than the LC-MS/MS methods with the highest GABA sensitivity described so far.^[1,2] Application of the established LC-MS/MS method indicated that HEK293 as well as COS-7 cells show significant endogenous GABA levels in comparison with the [3 H]GABA amount transported.

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P171

Synthesis and Biological Evaluation on New N-Substituted Noscopine Analogues

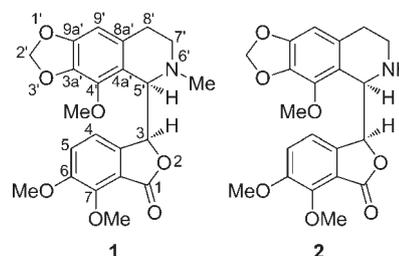
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The antitussive drug noscopine (**1**), isolated from *Papaver somniferum*, is a potential anticancer microtubule-binding agent.^[1] The rational structure-based screening of naturally derived compounds sharing structural similarities with toxic microtubule depolymerising agents such as colchicine and podophyllotoxin demonstrated noscopine to possess both the structural and binding similarities of these previously known microtubule depolymerizing compounds.^[1]

The structural modification of the noscopine scaffold,^[1] resulted in the synthesis of 9'-halo-substituted analogues: 9-fluoro-noscapine, 9-chloro-noscapine, 9-bromo-noscapine and 9-iodo-noscapine, which possess higher binding affinities for tubulin compared to noscopine.^[2] The 9-halogen substituted analogues showed a pronounced increase in the inhibition of proliferation of cancer cells compared with noscopine.

Herein, we report the efficient synthesis of *N*-noscopine and the subsequent reduction to the cyclic ether *N*-noscopine scaffold **2**. To further investigate the structure-activity relationship of *N*-substituted analogues, the reaction of cyclic ether *N*-noscopine, with suitable alkyl halides, acid chlorides, isocyanates and thioisocyanates, resulted in the synthesis of a number of *N*-alkyl, *N*-acyl, *N*-carbamoyl and *N*-thiocarbamoyl, cyclic ether analogues, which were pharmacologically evaluated against a number of cancer cell lines.



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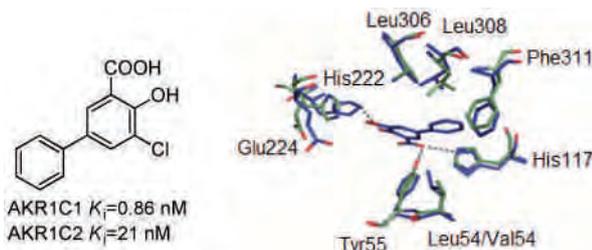
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Design and Synthesis of 20 α -Hydroxysteroid Dehydrogenase (AKR1C1) Inhibitors

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20 α -Hydroxysteroid dehydrogenase (20 α -HSD) is responsible for the pre-receptor regulation and modulation of steroid compounds in humans. It is one of four aldo-ketose reductase isozymes, which in humans share >80% homology. AKR1C1 (20 α -HSD) and AKR1C2 (type 3 3 α -HSD) differ in only seven amino acids yet display considerable substrate specificity.^[1] 20 α -HSD is primarily attributed to the metabolism of progesterone into the inactive progestin, 20-hydroxyprogesterone. Aberrant levels of this progestin have been associated with premature births leading to infant morbidity and mortality. 20 α -HSD has also been found to modulate the occupancy of γ -aminobutyric acid type A (GABA_A) receptors in the brain. Consequences of the inactivation of neuroactive steroids by 20 α -HSD have been affiliated with such neurological disorders as depression. Recent data suggests, 20 α -HSD is involved in the growth of several human and rodent tumours including endometrial, oesophageal, ovarian and breast cancers.^[2] The overexpression of 20 α -HSD in cancer cells is thought to be responsible for drug-resistance of several anticancer agents.^[3]



We identified from a virtual screening-based study several potent inhibitors of AKR1C1 that were subsequently used as lead compounds in drug design. Several new inhibitors have been synthesised based on the 3D structure, to which the potent compound, 5-phenyl-3-chlorosalicylic acid ($K_i=0.86$ nM) was identified.^[4] The project involves optimizing inhibitors to increase selectivity for the AKR1C1 isoform over the closely related AKR1C2 isozyme by targeting a non-conserved hydrophobic binding pocket.

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P173

Design, Synthesis and Biological Evaluation of D-Ring-Removed Estradiol Analogues as Subtype-Selective Estrogen Receptor Modulators

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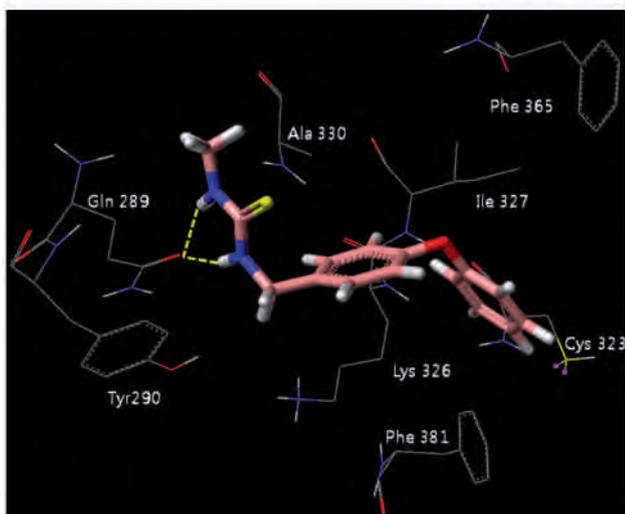
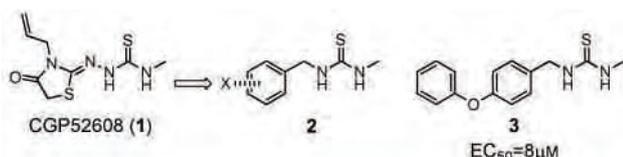
Estrogen receptor (ER) is a ligand activated transcription factor that belongs to the steroid hormone receptor family. They mediate the activity of estrogen, a hormone important for the development, maintenance, and regulation of the female reproductive system. ER is comprised of two subtypes, ER α and ER β . Studies on the tissue distribution of the two receptors show that they are widely and to a large extent differentially expressed in humans. For instance, ER β is largely expressed in the lung, prostate, and the brain, while ER α is predominant in uterus and breast. This observation suggests that the biological roles of ER α and ER β receptors might be tissue specific and that an ER subtype-selective ligand might produce a biological response which is different than the nonselective ligand 17 β -estradiol. These findings prompted the intensive efforts to develop the subtype-selective ligands acting on ER. Such ligands might have considerable potential for the treatment of a number of symptoms and/or diseases associated with estrogen deficiency, including hot flashes, osteoporosis and cardiovascular problems. More work is still needed to develop a novel selective estrogen receptor modulator (SERM) with improved antagonist effects in breast and uterus and robust agonistic actions in the skeletal, cardiovascular, and CNS. Searching for selective agonists having the new scaffold, we have designed and synthesized D-ring-removed estradiol analogues as subtype-selective ER agonist.

P174

N-Methylthioureas as New Agonists of Retinoic Acid Receptor-Related Orphan Receptor α

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Retinoic acid related-receptor orphan receptors α (ROR α) has been regarded as critical factors in the regulation of a number of physiological processes. The receptor plays an important role in the development of the cerebellum, lipid and steroid metabolism, hepatic lipid metabolism, homeostasis of cholesterol. Since those physiological functions of ROR α can be possibly modulated by exogenous ligands, the discovery of new non-natural ligands might lead to the development of novel therapeutics for human diseases that involve ROR α . In 1996, the thiazolidinone-type compound CGP52608 (**1**) were identified as efficient agonists of ROR α and showed antiarthritic activity in vivo. As part of our program to develop novel drug-like ROR α agonists for the treatment of metabolic disorders, we chose the first non-natural ligand, CGP52608, as a lead compound and attempted to replace the thiazolidin-4-one moiety with phenyl rings substituted with various functional groups. In this poster, we report the synthesis and ROR α activity of thiourea derivatives. Thirty-two N-methylthiourea derivatives (**2**) were easily prepared in one step from the corresponding amines or aldehydes, and their agonistic activities against ROR α were evaluated. Among them, 1-methyl-3-(4-phenoxy-benzyl)-thiourea (**3**) showed the best agonistic activity.

We believe this pharmacophore information would be very useful in the design of more potent agonistic scaffolds for the treatment of metabolic disorders such as fatty liver diseases.

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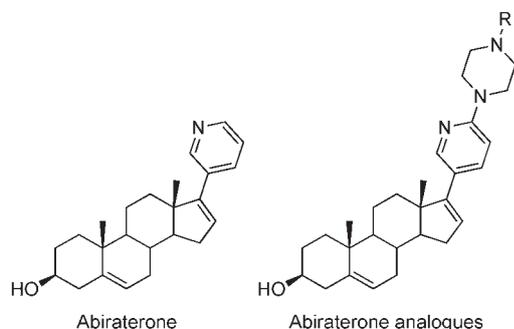
P175

Synthesis of Abiraterone Analogues, Their Effect on Cytochrome P450 CYP17A1/CYP19 and Antiproliferative Activities on Human Prostate and Breast Cancer Cell Lines

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Despite improvements in diagnosis and treatment, prostate cancer remains the second most common cause of death after lung cancer for men worldwide.^[1,2] It is well known that androgens and estrogens play an important role in the maturation of hormone-dependent cancers. Cytochrome P450 or CYP enzymes (CYP17A1 and CYP19) are involved in their biosynthesis. Recently, abiraterone (17-(3-pyridyl)androst-5,16-dien-3 β -ol) was developed as a highly selective and irreversible inhibitor of CYP17A1. Abiraterone acetate is currently in phase III clinical trial for men with castration-resistant prostate cancer.^[3] However, it has been shown that 33% of patients developed a resistance towards abiraterone.^[4,5] Similar natural molecules of this hybrid heterocycle-steroid are steroidal alkaloids that have shown a wide range of biological activities. Nevertheless, steroidal alkaloids and analogues remain poorly described in the literature. For these reasons, we thus thought that it would be fruitful to develop new abiraterone analogues where the pyridyl group is substituted with piperazinyl derivatives.



In this work, nine abiraterone analogues were synthesized and their inhibitory activity toward CYP17A1 and CYP19 was evaluated. Additionally, these heteroaryl steroids were tested on two human hormone-dependent cancer cell lines (prostate cancer and breast carcinoma cell line). These molecules were also tested on two hormone-independent prostate cancer cell lines. Among all tested compounds, three have shown a potent antiproliferative effect at 10 nM on hormone-independent prostate cancer cell lines with 60–85% inhibition of both cell viability and proliferation growth inhibition. Our data show that these molecules could be good leads in the design of drugs against both hormone-dependent and hormone-independent cancers by altering, respectively CYP17A1/CYP19 activities and cell proliferation.

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Renewable Nano Triterpenoids as Carriers for Anticancer Drugs

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Different types of drug delivery systems based on polymeric micelles, macromolecules, and nanoparticles have been designed to improve the pharmacological and therapeutic properties of drugs.^[1] However, development of a suitable drug delivery system still remains as an active area of investigation for biocompatibility as well as effective targeting of the delivery agent.^[2,3] Detailed computations carried out by us on 60 representative naturally occurring triterpenoids have established that all the triterpenoids are of nanometric lengths rendering them useful as renewable functional nano-entities.^[4]

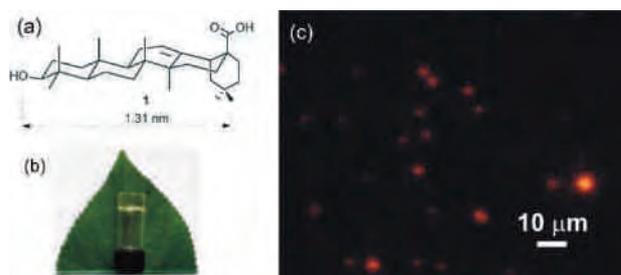


Figure 1. a) oleanolic acid **1**, b) a gel of oleanolic acid **1**, c) epifluorescent microscopy image of a solution of oleanolic acid in aqueous DMSO (2.2:1) containing doxorubicin drug.

We have initiated a long term project to utilize such renewable nanos in the design of nano-architectures and functional nanomaterials.^[5] Self-assembly studies of the renewable nanos in different liquids have shown that the molecules self-assemble in organic media to form nano-sized vesicles and helical nanofibers with concomitant hardening of the media (Figure 1b).^[6] The vesicular aggregates formed were capable of encapsulating drug molecules like doxorubicin in aqueous solvents, making it useful as a vehicle for drug delivery (Figure 1c).^[7] Recent results from our laboratory will be presented in the perspective of Green, Renewable and Nanos.

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P177

Synthesis of 6-Azapurines and their Nucleosides by Ring Transformation of 7-Azapteridines as Potential Antitumor Activities

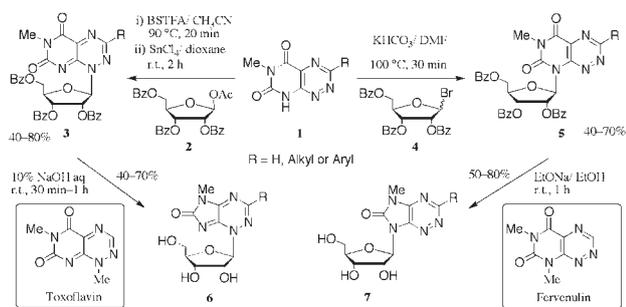
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Since the isolation and characterization of the naturally occurring antibiotics of 7-azapteridines, e.g. toxoflavin, fervenulin and reumycin (**1**: R=H) from *Pseudomonas cocovenenace*, *Streptomyces fervens*

n. sp. and *Actinomyces*, respectively, 7-azapteridines have been the subject of great deal of synthetic study, because of their marked biological activities.^[1] We have recently reported that the regioselective alkylations of **1** under alkaline conditions with a dialkyl sulfate or alkyl halide in dioxane or DMF to provide the 1-alkyltoxoflavins or 8-alkylfervenulins.^[2]

We herein report the regioselective glycosylation of reumycins (**1**) reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (**2**) and BSTFA in acetonitrile at 90°C followed by reaction of SnCl₄ in dioxane at room temperature afforded the 1-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-6-methylpyrimido[5,4-*e*][1,2,4]-triazine-5,7(1*H*,6*H*)-diones (**3**, toxoflavin-type nucleosides), while similar alkylations with 1-bromo-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (**4**) and K₂CO₃ in DMF at 100°C gave predominantly the 8-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-6-methylpyrimido[5,4-*e*][1,2,4]-triazine-5,7(6*H*,8*H*)-diones (**5**, fervenulin-type nucleosides). Moreover, we report the preparation of 1-(β -D-ribofuranosyl)-5-methyl-1*H*-imidazo[4,5-*e*][1,2,4]triazin-6(5*H*)-ones (**6**) and 7-(β -D-ribofuranosyl)-5-methyl-5*H*-imidazo[4,5-*e*][1,2,4]triazin-6(7*H*)-ones (**7**, 6-azapurine nucleosides) by benzilic acid rearrangement of **3** (toxoflavin-type nucleosides) and **5** (fervenulin-type nucleosides) in alkali solution, respectively. Their antitumor activities will be also discussed.



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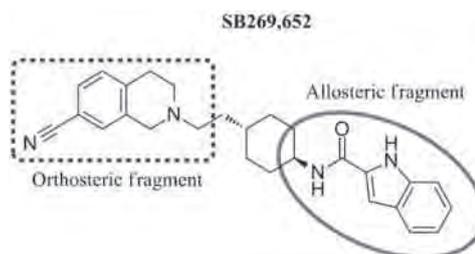
Identification of Bitopic and Allosteric Ligands Targeting the Dopamine D₂ Receptor

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The dopamine D₂ receptor (D₂R) has been strongly implicated in a number of disease states, including schizophrenia. Currently, most clinically available antipsychotic medications operate via orthosteric antagonism of the D₂R, and therefore compete directly with the

endogenous ligand, dopamine. Such an approach is associated with side effects, such as extrapyramidal symptoms and tardive dyskinesia. An alternative approach is negative allosteric modulation of the D₂R. Advantages associated with this approach may include greater receptor subtype selectivity due to lower allosteric binding-site homology across receptors compared to the orthosteric binding site, and an improved safety profile due to saturability of effect. We have confirmed and quantified the ability of SB269,652 to act as the first drug-like allosteric modulator of the D₂R.^[1] To investigate the mode of interaction of this ligand with the D₂R, we synthesised progressively truncated derivatives of SB269,652, which revealed a series of purely orthosteric antagonists (based on tetrahydroisoquinoline core), and a series of pure negative allosteric modulators (based on indole core) of the D₂R. This result indicates that SB269,652 is, in fact, the first bitopic ligand at the D₂R, with a dual orthosteric/allosteric binding mode. Furthermore, the identification of purely allosteric modulator fragments derived from SB269,652 represent a good starting point for the development of novel allosteric modulators of the D₂R; an approach that has yet to be exploited for the treatment of schizophrenia.



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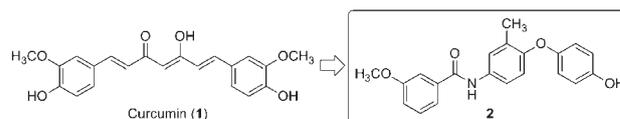
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P179

Development of Novel AR Antagonists Based on the Structure of Curcumin

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Androgen receptor (AR) is a member of the nuclear receptor superfamily of ligand-dependent transcriptional factors. Since AR is closely related to progression of prostate cancer, AR antagonists are clinically used for treatment of prostate cancer. However, chronic treatment of AR antagonists often causes hormone-refractory prostate cancer, and development of AR antagonists bearing novel pharmacophore has been desired. Almost all of the developed nonsteroidal AR an-

tagonists have a cyanophenyl group or nitrophenyl group as a pharmacophore, and therefore we investigated the development of novel AR antagonist bearing different pharmacophore.

Recent studies showed curcumin (**1**) and its derivatives have AR antagonistic activity,^[1] and we focused on **1** as a lead compound for development of novel AR antagonists. We have assumed that one of the phenolic hydroxyl groups of **1** is necessary for AR binding affinity, and have designed and synthesized various benzamide derivatives bearing the terminal phenol group. Biological evaluation using androgen-dependent SC-3 cells revealed the synthesized compounds exerted AR antagonistic activity. Among the synthesized compounds, compound **2** exhibited most potent AR antagonistic activity. Compound **2** also exhibited potent binding affinity to hAR and anti-androgenic activity toward human prostate cancer cell line LNCaP bearing T877A mutated AR. Compound **2** is a promising AR antagonist for development of AR pan-antagonists effective for hormone-refractory prostate cancer. A detailed synthesis and structure-activity relationship will be discussed.

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P180

Design and Synthesis of Aurones as Acetylcholinesterase Inhibitors

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Sulfuretin, a kind of aurone flavonoid derivative, is present in the lacquer tree, *Rhus verniciflua*,^[1] It has been known to possess diverse biological activities including antioxidant, antidiabetic, antimutagenic, antinociceptive and anti-inflammatory activities.^[2] In an effort to identify new structures of compounds to treat Alzheimer's disease, sulfuretin was found to possess acetylcholinesterase inhibitory effect although its potency is marginal ($IC_{50}=699 \mu M$). Accordingly, we synthesized various aurone derivatives using sulfuretin as a hit compound to increase acetylcholinesterase inhibitory activities. More specifically, we introduced hydroxyl, methoxy, or aminoalkoxy substituent at the aurone structure. Most compounds showed varied but more potent acetylcholinesterase inhibitory activities than sulfuretin. Of the synthesized compounds, aminoalkoxy-substituted aurone **2a** showed the most potent inhibitory activity with an IC_{50} value of $0.7 \mu M$, and its potency was much higher than

that of galantamine ($IC_{50}=4.7 \mu M$). The evaluation study for ameliorating effects of **2a** on scopolamine-induced memory impairment in mice is in progress.

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P181

SDOVS: A Solvent Dipole Ordering-Based Method for Virtual Screening

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Solvent dipole ordering (SDO) is an entity that captures an aspect of hydration structure.^[1] In our previous report, we have shown that SDO at the ligand binding site outlined the preferred shape and binding pose of the ligands, and pseudo-molecules that mimic the shape of the SDO region had a potential to screen active ligands for a target protein.^[2] In this work, we present a new virtual screening method based on SDO, referred to as SDOVS.

The general procedure of SDOVS is as follows:

- 1) Perform MD simulation for the target protein and obtain SDO.
- 2) Define SDO region according to desired MW range of ligands.
- 3) Generate the pseudo-molecules that mimic the shape of the SDO region.
- 4) Screen similar compounds to the pseudo-molecules from compound DB with multiple conformers.
- 5) Perform geometry optimization of the compound in the protein and calculate interaction energy.
- 6) Check the hydrogen bonds and select one conformation with the lowest energy.

This method was applied to four typical drug target proteins and compared the performance with FRED, a well-known rigid docking tool. As a result, SDOVS could obtain more diverse compound structures than FRED. Examples of overlays of a pseudo-molecule with a selected compound and the shape Tanimoto scores are shown in Figure 1. The advantages of this method are: 1) SDO cover whole ligand binding site, 2) easy to obtain flexible compounds, 3) applicable without real active molecules, and so on.

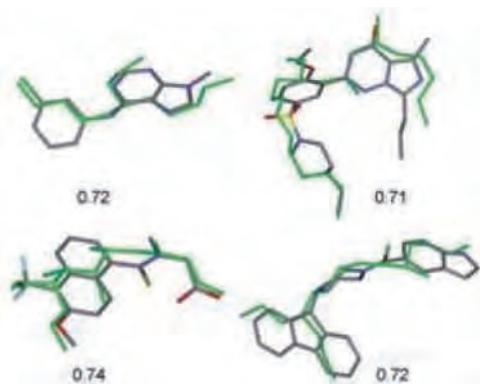


Figure 1. Overlays of a pseudo-molecule with a selected compound and the shape Tanimoto scores.

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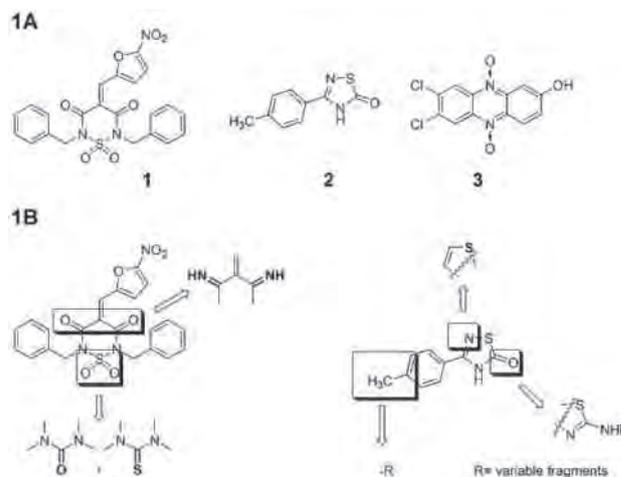
P182

Tc-TIM Hits—Structural Modifications Looking for Leads with Anti-*T. cruzi* Activities

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Recently, we have identified new structural hits with capability to irreversibly inhibit *Trypanosoma cruzi* triosephosphate isomerase (Tc-TIM) dimer-interface.^[1] Additionally, the capability to inhibit *homo sapiens* TIM has been analyzed finding that the most selective compounds, SI>4, have been **1**, **2**, and **3** (Figure 1A).^[2] Except for compound **3**, the capability to inhibit the parasite growth has been very scarce. For this reason, we have planned a series of structural modifications on hits **1** and **2** in order to improve the activities against the whole parasite without loss of anti-Tc-TIM activity (Figure 1B).



Different series of compounds have been synthesized, biological evaluated against whole parasite (*T. cruzi* epimastigotes), and for the best parasite growth inhibitors Tc-TIM inhibition capabilities have been studied.

One of the new thiazole derivatives has displayed excellent activity against the parasite and according its structure could inhibit Tc-TIM covalently.

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P183

Using Rational Drug Design of beta-Secretase Inhibitors in Alzheimer's Disease

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Alzheimer's Disease (AD) is the major cause of senile dementia, effecting up to 37 million patients in 2010.^[1] The disease causes a neurodegeneration in the hippocampus, basal nucleus, associative and entorhinal cortex, resulting in cognitive impairment, physiological dysfunctions, memory loss and in advanced cases, catharses and death.^[2]

The beginning of the neurodegenerative process is attributed to the excessive production and accumulation of an amyloid peptide with 40/42 residues (A β) in intracellular oligomers and extracellular senile plaques.^[3] The exact mechanism of how the A β leads to the neurodegeneration is still unclear, but it is known that the inhibition of its production blocks disease progression.

In this context, β -secretase 1 (BACE-1), a transmembrane aspartic protease, plays an important role once the beta-amyloid component of plaques is produced by cleavage of the amyloid precursor protein (APP). BACE-1 inhibition is considered one of the most promising alternatives to AD treatment, since the current drugs do not break such progress.^[4]

In this work, different virtual screening experiments were performed with GOLD, GLIDE (docking approaches) and Discovery Studio (pharmacophore-based approaches), using the MayBridge, Chembridge and ZINC (CNS collection) databases to select compounds with good in silico binding affinity for the β -secretase catalytic site. These compounds were further evaluated in phase with Molecular Interaction Fields maps, which were produced using the NH₂, O₂, OH and aromatic probes. For ligand-based drug design, we used three different and selected 4-featured pharmacophore models, which represent the most important interactions of ligands with residues of the BACE-1 active site, in special the catalytic aspartates and the ones of the "flap" hairpin. A final selection of several amongst thousands of compounds included in silico toxicity and activity analyses.

The consensus results obtained from such different methodologies reveal novel promising compounds that will be further evaluated by molecular dynamics and in vitro assays regarding its β -secretase inhibitory activity.

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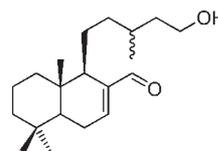
P184

Initial Preclinical Studies of a Natural Labdene with In Vitro Anti-*T. cruzi* Activity

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Recently, we have isolated from the aerial parts of *Aristeguetia glutinosa* Lam. (+)-15-hydroxy-7-labden-17-al (shown), which has displayed excellent in vitro activity against *Trypanosoma cruzi*, the etiologic agent of Chagas disease.^[1] To explore the potential of the initial extract, sub-extracts or isolated compound as drugs, we have performed different preclinical studies. Firstly, we have evaluated the safety analyzing capability to produce red blood cells lysis, unspecific macrophage cytotoxicity, and mutagenic capacity by Ames test (*S. typhimurium* procedure). Secondly, we have completed the proof of concept in animals using a murine model of Chagas disease. In these studies, we have used the oral administration of initial extract or isolated compound, and the parasitemia, antibodies levels and organs histopathology as findings.



Finally, in order to determine the labdene mechanism of action, different experiments have been performed. We have studied the effect of studied compound on the membrane sterol biosynthesis,^[2] on the mitochondrial dehydrogenase,^[3] and on the excreted metabolites. Additionally, we have studied the type of cellular death promoted by the compound using ¹H NMR spectroscopy.^[4]

The excellent results with (+)-15-hydroxy-7-labden-17-al support the vernacular medicinal use of *Aristeguetia glutinosa* Lam. as an anti-*T. cruzi* agent.

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P185

Positive Allosteric Modulators of the M₄ Muscarinic Acetylcholine Receptor

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There are currently five known subtypes of muscarinic receptors (mAChR), M₁–M₅, all of which belong to the superfamily of GPCRs. The M₄ mAChR has been implicated in several CNS disorders, particularly schizophrenia^[1] and therefore presents an alternative approach to alleviating the symptoms associated with this disorder. However, due to the high amino acid conservation of the orthosteric site between each of the five subtypes, efforts towards discovery of target selective agonists have been impeded. Fortunately, like many GPCRs, the M₄ mAChR possesses a secondary binding site, topographically distinct from the orthosteric site, called the allosteric site, for which several allosteric ligands have already been identified. Such allosteric ligands have shown abilities to positively modulate the affinity and/or efficacy of the endogenous ligand (ACh).^[2] The synthesis of putative M₄ PAMs was carried out based on a bicyclic scaffold (Figure 1) identified by Shirey et al.^[3] and Brady et al.^[4] We have modified the right-hand side of the VU lead compound to afford a focused library of compounds that investigate the electronic and positional effects of some favourable substituents. The synthesised compounds were further evaluated pharmacologically providing useful estimates of affinity, cooperativity and agonist-like properties. These data have generated an ‘enriched SAR’ profile for the compound series and resulted in the identification of several potential M₄ PAMs.

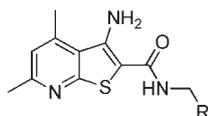


Figure 1. Bicyclic scaffold of positive allosteric modulators of the M₄ mAChR.

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P186

Detecting Conformational Changes in Protein Kinases with an HTS-Compatible Assay

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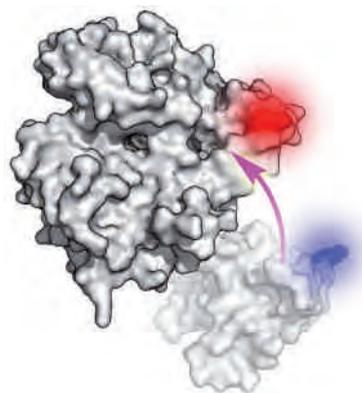
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Protein kinases are key components of cellular signalling pathways, so misregulation of kinase activity can be the cause of various diseases including cancer. A large number of protein kinases are regulated by changes in conformation and complex assembly. These intricate mechanisms are often triggered and regulated by protein-protein interactions. Across the kinome, they are highly diverse and often characteristic for a particular class of protein kinases.

Small-molecule inhibitors that specifically address these interactions and stabilise enzymatically inactive conformations have shown superior selectivity over traditional ATP-competitive inhibitors, owing to their allosteric mode of action. Approaches that allow for the unambiguous identification of such allosteric inhibitors have fallen short so far.

Here we report, for the first time, the development of a fluorescence-based kinase assay, which takes advantage of inhibitor-induced structural changes of protein kinases and, in particular, reports on inter-domain crosstalk.



P187

Lead Optimization in a Novel Class of Gamma Secretase Modulators

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Gamma secretase produces amyloid beta peptides of varying lengths by processing the C-terminal fragment of amyloid precursor protein. Studies have implicated these A β proteins, particularly A β 42, as playing a key role in the pathogenesis of Alzheimer's disease. Satori has discovered a unique class of small molecules capable of modulating gamma secretase such that the distribution of A β polypeptides is shifted away from amyloidogenic A β 42 to shorter species without reducing the total A β pool. This presentation will describe the lead optimization program that transformed early compounds with promising pharmacology to those which possessed superior in vivo performance. This was accomplished, in part, by replacing pharmacologically relevant pharmacophores with bioisosteres that improved the overall physicochemical properties of the molecules, thereby reducing clearance and improving in vivo disposition.

P188

Overcoming Gatekeeper Mutations in Kinases by Hybrid Compound Design

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The majority of commercially available anticancer drugs that target protein kinases bind within the ATP pocket of the catalytic domain (type I inhibitors). Although such inhibitors are very successful in treating the early stages of disease control in some patient populations, the emergence of drug-resistance is becoming an ever-increasing challenge. One of the most prominent drug resistance mutations is the replacement of the gatekeeper residue in the hinge region of the kinase domain with a bulky and often aliphatic amino acid (e.g., in Bcr-Abl_T315I and cSrc_T338M). Current efforts in kinase inhibitor research focus on overcoming these mutations by developing inhibitors which a) bind exclusively outside the ATP pocket and b) lock the kinase in an enzymatically inactive conformation.^[1,2]

We recently designed and synthesized type II kinase inhibitors active against the drug-resistant mutant variant cSrc_T338M by fusing fragments of type I and III inhibitors.^[3] Here, we report on the enhancement of these inhibitors, their potency against drug resistant Abl_T315I in biochemical and cellular assays as well as their crystal structures in complex with cSrc. To further explore the structural features responsible for potency and selectivity of these hybrid inhibitors, we performed affinity chromatography by immobilizing inhibitor fragments to solid support in order to pull down target proteins from K562 cell lysates. Subsequent mass spectrometry analysis was then used to elucidate the kinase targets of the fragments.

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P189

Flavors of Discovery: Computational Predictions of New Agonists of the Bitter Taste Receptor hTAS2R14

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Bitter taste is a basic taste modality required to guard animals against consuming toxic substances. Bitter compounds are recognized by bitter taste receptors (TAS2Rs), a family of G-protein coupled receptors (GPCRs). The human bitter taste receptor hTAS2R14 is a particularly broadly tuned receptor with over 50 agonists known to date. Analysis of the physicochemical properties of these molecules in comparison with true negatives—i.e., molecules known not to activate hTAS2R14—provided hTAS2R14-characteristic ranges of chemical properties.

To identify additional potential agonists of this receptor, we compiled a pool of candidate molecules, consisting of the established bitter-tasting compounds from the BitterDB database, and other potentially bitter molecules, such as datasets of approved drugs, traditional Chinese medicines and natural compounds. This dataset of candidate molecules was filtered using the hTAS2R14-like property ranges, resulting in a subspace of candidate molecules that could potentially activate hTAS2R14.

Next, ligand-based and structure-based pharmacophore models of hTAS2R14 activators were constructed and used to prioritize the candidate subset. Preliminary results using functional assays of hTAS2R14-transfected HEK293 cells confirm that most of the predicted substances are indeed novel hTAS2R14 agonists.

This approach provides new directions in the identification and design of agonists and antagonists for bitter taste receptors, as biochemical tools for studying these receptors, and for improvement of food taste. Importantly, the recently discovered roles of bitter taste receptors in extraoral locations, such as the respiratory and gastrointestinal systems, provide novel paths of drug design for treatment of metabolic disorders and other indications.

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P190

Identification of a New Chemical Class of Potent Antimalarial Compounds

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Multitarget antimalarials, characterized by a 4,4'-oxybisbenzoic acid linker between statine, a plasmepsins inhibitor, and primaquine were previously synthesized.^[1] SAR-driven improvements of efficacy and pharmacology indicated that the 4,4'-oxybisbenzoic acid linker bound to two amino acids (leucine, isoleu or alanine only), is the minimal structural feature to retain antimalarial activity. A series of molecules characterised by the presence of this novel chemotype were synthesised and showed $IC_{50} < 15$ nM against drug resistant *Pf* in vitro, no toxicity against, no inhibition of plasmepsins, or of β -haematin formation. A systematic study was performed to reduce the MW and improve the metabolic stability while retaining potency and selectivity. Critical structural features such as the ester function and the two alkyl-branched amino acids were modified by replacing the flexible substituents with rings to reduce the number of rotatable bonds and the ester with more stable functions, and by substitution of amino acids with drug-like scaffolds. A series of hits was generated with high activity in vitro ($IC_{50} < 1$ nM) against synchronized ring-stage parasites. These characteristics: new chemotype, high selectivity, low toxicity and fast action suggest that this new chemotype could represent a new lead which adheres to the target product profile needed for elimination (and ultimately for eradication) of malaria.

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P192

From Peptidomimetics to Nonpeptidic BACE-1 Inhibitors through the Fragment-Based Drug Design Technique

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We have previously reported BACE-1 inhibition by hydroxyethylamine (HEA) and hydroxyethylsulfide (HES) transition-state isosteres. These peptidomimetics inhibitors were very potent in blocking the proteolytic activity of BACE-1, but deprived of activity in the cell-based assays. We showed that while the *syn* isomer of HEA inhibitors was preferred by BACE-1, the stereo preference of HES inhibitors was opposite, thus the *anti* isomer resulted more active. This peculiar change in stereo preference was explained by molecular modeling studies.

Considering the therapeutic necessity of new molecules, we focused our attention in designing selective BACE-1 inhibitors with good pharmacokinetics and pharmacodynamics properties able to cross the blood–brain barrier. A new strategy for the design of novel drug-like inhibitors was therefore employed. Fragment-based drug design is an efficient and productive route for drug discovery, since it uses sets of drug-like chemical fragment and the 3D structure of biological target. This technique allows high-quality drug-like molecules to be obtained, despite the multiplicity of combined parameters. This project was developed using commercially available computational chemistry software and molecular modeling programs with the existing BACE-1 crystal structure.

After these studies, the designed molecules were synthesized and hereby we introduce new potential BACE-1 low-molecular-weight inhibitors.

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Solid-Phase-Supported Mukaiyama Reagent as an Efficient Tool for the Synthesis of Enantiomers of Cyclic Amino Acids

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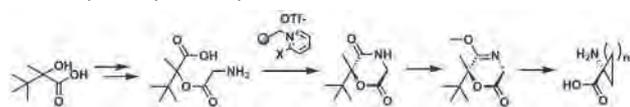
Conformationally constrained amino acids (AAs) have been the focus of both synthetic and medicinal chemistry, particularly as they apply to the design of novel peptides. Rigidified cyclic amino acids (CAAs) have also played an important role in drug design and development, where they exert conformational constraints while maintaining the hydrophobic character of the linear alkyl chains.

Thus, the incorporation of CAAs into peptides or peptidomimetics induces conformational restrictions and provides important structural effects.^[1]

During our study, we focus on the elaboration of convenient methods of the synthesis of enantiomers of cyclic amino acid using enantiomers of glycine equivalent described by Wanner and co-workers.^[2] However, the most crucial point in the synthesis of this glycine equivalent is cyclization of 2-(2-aminoacetoxy)-2,3,3-trimethylbutanoic acid, which is performed using Mukaiyama reagent (2-chloro-1-methylpyridinium iodide).

Mukaiyama reagent has been extensively used as an acid-activating agent, but its insolubility and the side products of the reactions resulted in low effectiveness of this process.

Thus, based on a literature survey, it was decided to replace classical Mukaiyama reagent by a solid-phase-supported one. Such modification resulted in significant increased yield of this reaction and simplified product purification.



Acknowledgements: The financial support of this work by the Jagiellonian University Medical College grant for young scientist is gratefully acknowledged.

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P194

LASSBio-596: A Symbiotic Antiasthmatic Prototype

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Multifactorial degenerative diseases, e.g., asthma, cannot be effectively treated with single-target drugs. Current therapeutic approaches for the treatment of asthma use drug associations, indicating that the successful treatment is based on a pharmacological intervention in more than one molecular target, which can be achieved by the so-called symbiotic drugs. Symbiotic agents are prototypes designed to act on at least two different molecular targets belonging to distinct biochemical routes, however, related to the same disease.^[1]

The Brazilian National Institute for Science and Technology in Drugs and Medicines (INCT-INOFAR, <http://www.inct-inofar.ccs.ufrj.br>, CNPq BR #573.564/2008-6) connects a network of researchers with expertise in different areas sharing the common goal of innovation and drug discovery. Among the research topics, INCT-INOFAR highlights the search for a new symbiotic antiasthmatic prototype. In this context, the Laboratory of Evaluation and Synthesis of Bioactive Substances (LASSBio®, UFRJ-BR, <http://www.farmacia.ufrj.br/lassbio/>) has previously described **LASSBio-468** as a novel dual-target anti-inflammatory lead compound, acting as TNF- α and PDE-4 inhibitor.^[2,3]

LASSBio-468 is an achiral phthalimidic derivative, which can be synthesized in good overall yield on a 0.5 M scale.^[2] Knowing that the phthalimidic drug thalidomide undergoes non-enzymatic hydrolytic cleavage (pH 7.4), resulting in partial hydrolysis of all imides present in its structure,^[4] the plasma and chemical stability of **LASSBio-468** were studied in order to check its metabolic lability. The results have shown that the phthalimidic core of **LASSBio-468** is labile to partial hydrolysis at pH 7.4 even in the absence of plasma hydrolases, generating the corresponding carboxamide **LASSBio-596**.

Considering the possibility that the pharmacological effects observed in vivo for **LASSBio-468** could result from its hydrolysis to **LASSBio-596**, this metabolite was synthesized and tested in a murine model of acute lung injury. **LASSBio-596** was able to modulate the pulmonary inflammatory process, reversing the mechanical alterations in the airways, blocking the fibroproliferation and in-

hibiting the neutrophil recruitment and the production of TNF- α .^[5] **LASSBio-596** was additionally able to prevent the morphologic and mechanical alterations in the airways in a murine model of chronic asthma.^[6]

The integrated efforts of the involved researchers, which began in the framework of the Millennium Institute for Innovation and Development of Drugs and Medicines (CNPq BR #420015/05-1) and are now continued in the INCT-INOVAR (CNPq BR #573.564/2008-6) enabled the discovery of a new antiasthmatic drug candidate, i.e. **LASSBio-596**, orally active in murine models of acute and chronic asthma. The bioavailability and safety profiles (i.e., genotoxicity, mutagenicity, acute and chronic toxicities in rodents) were also determined for this drug candidate.^[7]

Given the promising results presented for the antiasthmatic prototype, **LASSBio-596**, in the preclinical studies conducted so far, the INCT-INOVAR goes on with the preclinical studies, aiming finally to fulfill the regulatory requirements for the future clinical trials stage of phase I.

Acknowledgements: CNPq (BR), INCT-INOVAR (BR), FAPERJ (BR) and to Prof. Dr. Stefan Laufer (Eberhard-Karls-University Tübingen, Germany) for helpful discussions.

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Design of Novel Di-*tert*-butyl-phenol-morpholine Derivatives with Antihyperlipidemic, Potent Antioxidant and Antidiabetic Action

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Oxidative stress, inflammation and hyperlipidemia are common factors involved in the pathophysiology of atherosclerosis and type 2 diabetes. A therapeutic single-targeted approach for such multifactorial diseases is mostly considered inadequate. We have previously developed multifunctional antidiabetic morpholine derivatives with antioxidant and antiatherogenic properties.^[1-3] Further, succinobucol, an antioxidant di-*tert*-butyl-phenol derivative, designed as an antiatherosclerotic drug, has shown promising benefits in the prevention and treatment of type 2 diabetes.^[4] Thus, we proceeded to incorporate structural features of succinobucol in the pharmacophore of our antihyperlipidemic aromatically-substituted morpholines. These molecules were synthesized employing modifications of existing synthetic methodology and their structures were confirmed spectroscopically and by elemental analysis.

Designed to combine within one structure both antidiabetic and enhanced antioxidant properties, the new compounds were as such evaluated both in vitro and in vivo. They exhibited improved antioxidant activity: a) inhibition of Fe²⁺/ascorbate-induced lipid peroxidation of rat microsomal membranes with IC₅₀ values around 4 μ M, b) almost total inhibition of in vitro human LDL peroxidation in the presence of low concentrations of these molecules. The new compounds exhibited significant antihyperlipidemic effects in rat, reducing plasma levels of total cholesterol and triglycerides up to 90% and 76%, respectively. Compounds showed high antioxidant capacity also in vivo, reducing MDA plasma levels by 64%.

Subsequently, using a type 2 diabetes experimental animal model, via combination of a high fat diet and multiple low doses of streptozotocin, the most potent antioxidant/antidiabetic compound (designed to incorporate a structural moiety of the antidiabetic agent succinobucol) was evaluated for its antidiabetic activity. It produced a significant reduction of elevated blood glucose, body weight, total cholesterol, LDL cholesterol and MDA levels, while it increased blood HDL/LDL ratio.

Rational drug design led to a compound with improved antioxidant, antidiabetic but also antidiabetic action. This combination of activities within a single structure provides a unique starting point for the development of novel therapeutics for metabolic syndrome disorders.

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P197

Prodrug Approaches for the Neuraminidase Inhibitor Oseltamivir: Tackling Influenza A Resistance Along with Unfavorable Pharmaceutical Properties

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According to the WHO, the influenza virus is responsible for diseases of 3–5 million people and 250.000 to 500.000 deaths worldwide that are either caused directly by the virus or by secondary infections. The last pandemic occurred in 2009 (H1N1-virus, swine flu) but luckily turned out rather harmless regarding the number of deaths. In recent years the highly pathogenic H5N1-virus (bird flu) is particularly concerning, as it can be transmitted from animals to humans. Statistically, a new severe pandemic is overdue. A general issue with anti-influenza therapy is the high mutagenic rate of the virus, especially of its surface proteins hemagglutinin and neuraminidase, which contribute to the rapid development of resistance against currently approved drugs. Numerous resistances against amantadines have been described to date. Few are also reported already for oseltamivir, and this number is steadily increasing. Moreover, marketed neuraminidase inhibitors suffer from distinct pharmaceutical drawbacks, i.e. their oral bioavailability.

Our motivation was to design neuraminidase inhibitors that overcome both influenza A resistance and bioavailability issues, thereby tackling key problems associated with the current anti-influenza therapeutic regimen. With oseltamivir as the lead structure, we developed 5-amidino and -guanidino analogues that show comparable potency against a panel of different H3N1 and H1N1 influenza strains as well as efficacy against an oseltamivir-resistant H1N1 virus strain (Berlin/342/09). A series of prodrugs for these candidates were then evaluated for their in vitro and in vivo pharmacokinetic properties and turned out to exhibit profiles that are competitive with oseltamivir.

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2-Substituted *N*-Benzylamides of 4-Hydroxybutyric Acids, New GABA-Uptake Inhibitors

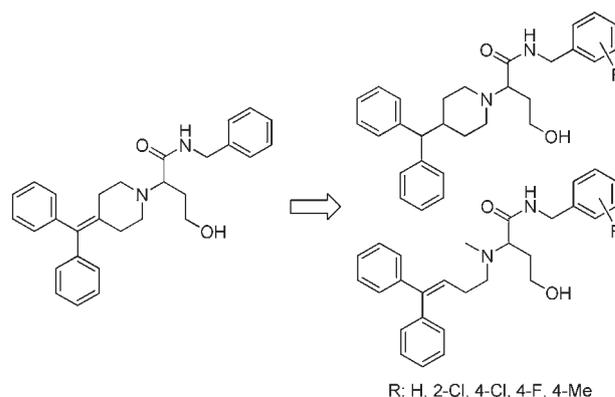
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4-Aminobutyric acid (GABA) is an inhibitory neurotransmitter, which is involved in the control of neuronal activity in the mammalian central nervous system (CNS). There is considerable direct and indirect evidence that impair activity of GABA-mediated inhibitory synapses might be an important causative factor in experimental and clinical seizure disorders. Since GABAergic neurotransmission is terminated by uptake into neuron or glia cells, inhibitions of GABA transporters responsible for uptake would prolong the GABAergic signal. Although many GABA uptake inhibitors possess antiepileptic properties, only tiagabine is GAT inhibitor currently available for the treatment of epilepsy and neuropathic pain.^[1]

Taking above into consideration and the interesting results of our earlier studies, a new series of *N*-benzylamides of 4-hydroxybutanoic acid (GHB) was designed and synthesized.^[2,3] The designed changes were focused on the structural modifications in the 2nd position of the GHB within the benzyl fragment of the molecule of *N*-benzylamide. The obtained compounds have been tested for their inhibitory potency at the four murine GABA uptake transporters mGAT1-mGAT4 stably expressed in HEK cells.



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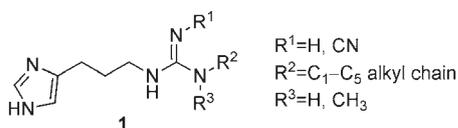
Derivatives of Imidazolylpropylguanidine (SK&F-91486): Synthesis and Some Pharmacological In Vitro Activities

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3-(1*H*-Imidazol-4-yl)propylguanidine (SK&F-91486,^[1] **1** [R¹=R²=R³=H]) is the long-known prototypic pharmacophore of highly potent histamine H₂-receptor (H₂R) agonists of the guanidine class of compounds including, e.g., arpromidine^[2] and the recently developed acylguanidines.^[3] In functional in vitro experiments, these agonists produced a chronotropic response in the isolated, spontaneously beating guinea-pig right atrium assay that was susceptible to blockade by cimetidine, a prototypic H₂R antagonist. However, in our hands, cimetidine and other typical H₂R antagonists (ranitidine, famotidine) were surprisingly unable to antagonise the positive chronotropic response elicited by SK&F-91486,^[4] although the compound so far has been unanimously classified as a weak partial H₂R agonist.

We studied the in vitro properties of SK&F-91486 in the guinea-pig atrium assay in more detail, and additionally found a similar behaviour for the 2-methyl derivative of SK&F-91486, and for guanethidine, another guanidine-containing drug molecule with a second basic moiety.



In order to gain more insight into the structure-activity relationships of simple analogues of SK&F-91486, we started a project aiming at the synthesis and in vitro characterisation of closely related imidazolylpropylguanidines. Starting from homo-histamine (obtained by a seven-step synthesis from *trans*-urocanic acid), cyanoguanidines were obtained using diphenylcyanocarbonimidate according to reported procedures.^[2,3] Treatment under acidic conditions led to the final guanidines. Alternatively, some compounds were obtained from benzoylthiocyanate via the respective substituted thioureas, S-methylation and final nucleophilic substitution with homo-histamine in the presence of HgCl₂ as catalyst.

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Multigram-Scale Synthesis and In Vivo Efficacy Studies of the Multitarget Anti-Alzheimer Compound AVCRI104P4

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Compelling evidence suggests that many common diseases do not result from a single abnormality but from multiple molecular defects. The use of compounds which simultaneously hit multiple molecular targets involved in the pathogenesis of a given disease should be associated with increased efficacy and safety relative to single-target therapeutic interventions. Alzheimer's disease (AD) is also a multifactorial disease which might benefit from a multitarget therapeutic approach.

Compound AVCRI104P4 has recently been found to exhibit a multitarget profile in vitro that encompasses inhibitory activities toward human cholinesterases, BACE-1, and beta-amyloid aggregation. Herein, we report on the scale-up of the synthesis of AVCRI104P4 to a multigram scale and preliminary in vivo preclinical studies in two different animal models of AD, namely APP_{SL} transgenic mice and *Caenorhabditis elegans* (CL4176 and CL2006 strains).

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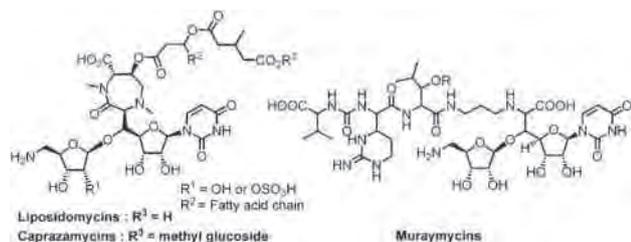
Analogues of Muraymycins as New Inhibitors of the Bacterial Transferase MraY

Christine Gravier-Pelletier, Mickael Fer, Sandrine Calvet-Vitale, Delphine Lecerclé

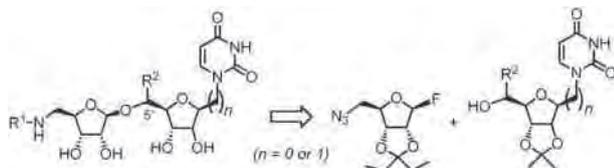
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The bacterial peptidoglycan is known to be an essential biopolymer of the bacteria membrane. It is both specific to bacteria and essential to their survival. Owing to their high specificity and their sole occurrence in bacteria, the enzymes implicated in peptidoglycan

biosynthesis are promising targets to discover novel antibacterial agents since their inhibition should permit to develop compounds nontoxic to mammals. In this context, we are focusing on inhibition of the *MraY* transferase,^[1] which catalyzes the first membrane step of peptidoglycan biosynthesis. Indeed, due to its trans-membrane localisation, it has been little exploited and it is currently the target of no antibiotics in clinical use. Several families of natural inhibitors of *MraY* are known, such as muraymycins, liposidomycins or caprazamycins, however they display limited antibacterial activity. The aminoribosyl uridine moiety is a common feature of these compounds and has been shown to be essential for *MraY* inhibition.



In the continuity of our program aiming at *MraY* inhibition,^[2-4] we are developing the synthesis of new inhibitors based either on an aminoribosyl-*O*-uridine like scaffold ($n=1$) and containing modifications on the amine function or on an aminoribosyl-*O*-uridine scaffold ($n=0$) with a free amine and various triazole-containing moieties at the 5' position. Depending on the substituent introduced on the triazole, the inhibitors can be used as chemical tools for *MraY* active site mapping.



The inhibitors synthesis and the results concerning their biological evaluation (UMR8619 CNRS Université Paris XI, Dr. A. Bouhss, et al.) will be discussed.

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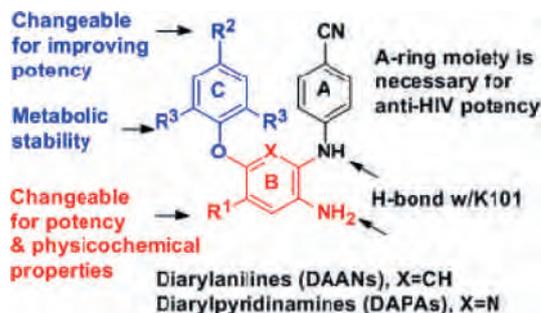
P205

Synthesis, Anti-HIV Activity and Drug-Like Property Evaluation of Diarylanilines and Diarylpyridinamines as Novel HIV-1 NNRTIs

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We have discovered two series of new diarylanilines (DAANs) and diarylpyridinamines (DAPAs) with nanomolar anti-HIV potencies against wild-type and HIV-1 RT-resistant viral strains.^[1-3] These compounds are promising new anti-AIDS drug candidates due to their high potency, new chemo-type scaffold, and simplicity of synthesis. To further improve the metabolic stability and aqueous solubility of these compounds, our lead optimization was focused on substituents on the central phenyl or pyridine ring (B-ring) and the tri-substituted phenoxy ring (C-ring) as shown in the figure below. As a result, a few dozens of new highly potent DAANs and DAPAs were synthesized. These new compounds inhibited HIV-1 at low nano- to sub-nanomolar concentration with EC₅₀ values ranging from 0.2 to 10 nM. These potent DAANs and DAPAs were further evaluated for their drug-like properties including aqueous solubility, metabolic stability,^[4] and their pharmacokinetic profiles in rats. Data from these preclinical pharmacokinetic and pharmacodynamics studies suggest that the new DAANs and DAPAs are promising drug candidates to be developed into a next-generation of HIV-1 NNRTI.



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P206

Pharmacophore Model Construction of p53–MDM2 Binding Inhibitors and its Application in the Discovery of a Novel Lead Compound

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The close association between p53 and cancer has been recognized for decades. And MDM2 is a main negative regulator of the tumor suppressor protein p53. Previous research has indicated that suppressing the tumor cells by inhibiting the binding of MDM2 to p53 with chemical compounds is a worthy endeavor as a new therapeutic strategy for cancer.^[1]

In hopes of identifying novel p53-MDM2 binding inhibitors with higher potency and lower toxicity, a pharmacophore model (Figure 1A) was set up based on the structures of reported p53-MDM2 binding inhibitors using Accelrys Catalyst package. The reliability of this model was confirmed by different parameters (Δ cost = 313.366, config = 17.024, corrol = 0.920) as well as hierarchical cluster analysis, activity-predicting ability test (corrol=0.822 for experimental activities against estimated activities of test set), CatScramble verification and enrichment factors (20 active and 1200 inactive molecules, 66.7, 18.8 and 8.6 at 2%, 5% and 10% respectively). It tells that three hydrophobic groups on the core structure are indispensable for a desirable p53-MDM2 binding inhibitor, and two aromatic rings are also of significant importance to the inhibitory activities. Feature mapping of the model and Nutlin 3, a potent p53-MDM2 binding inhibitor reported, exhibited good results both for the molecule along (Figure 1B).

Several hits were retrieved through virtual screening against NCI, MiniMaybridge and in-house databases using the established pharmacophore model. Following docking studies identified a 3,4,5-trisubstituted aminothiophene derivative (**1**, Figure 1C) as a lead compound targeting p53-MDM2 interaction. Biological evaluation reveals that compound **1** showed an IC₅₀ value of 4.2 μ M and K_i value of 1.1 μ M against p53-MDM2 interaction. Further design and in-depth investigation based on this lead is intensely undertaking and optimistic results have been continuously achieving.

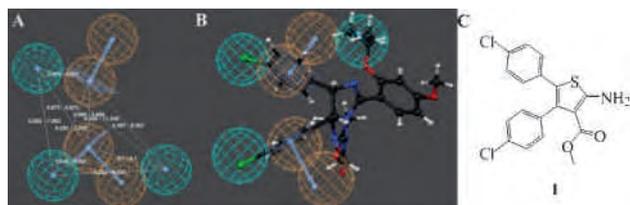


Figure 1. A) Established pharmacophore model with distance constraints. B) Feature mapping of model and Nut-3. C) Novel lead retrieved through virtual screening.

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P207

Single Molecule, Real-Time Observation of DNA Interacting with Dinuclear Platinum Antitumor Drugs

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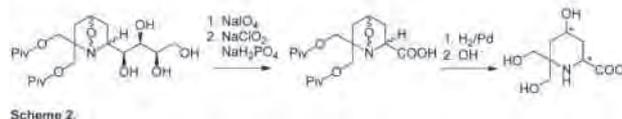
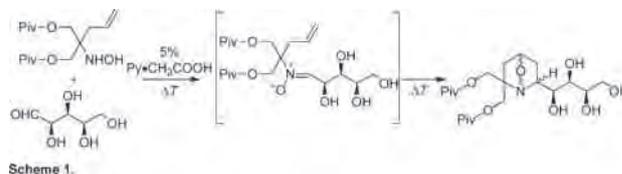
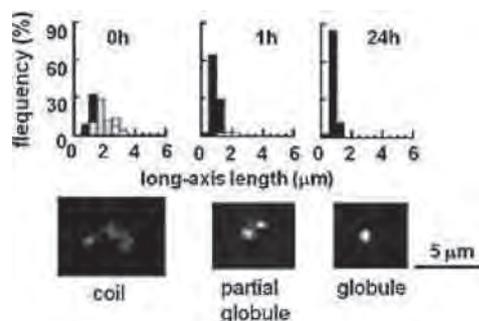
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Genomic DNA is the molecular target for many chemotherapeutic drugs in cancer treatments. Much attention has been focused on studying the interaction of drugs with DNA and developing new DNA-targeted drugs. As for the research of DNA–drug interactions, X-ray crystallography and NMR techniques are useful for defining the detail local binding mode of drug–DNA complex, but require the crystallization of DNA or encounter the limitation of size of DNA. Thus, these techniques are not adequate to investigate the effect of drugs on the overall morphology of a large DNA. Since genomic DNA is a very long polymer, studying the change of the higher-order structure of large DNA induced by drugs may provide additional insights for understanding the mechanism of their activities in living cells. Platinum compounds, including cisplatin, are now among the most commonly used anticancer drugs. Many studies have been conducted to understand the mechanism of action of cisplatin. It is generally accepted that cisplatin forms coordinative adducts with genomic DNA, such as 1,2-intrastrand cross-links, to interfere with transcription and/or DNA replication, which eventually leads to apoptotic cell death. Thus, the Pt–DNA binding modes and kinetics seem to be closely related to its anticancer activity. Despite the high potential anticancer activity of cisplatin, its clinical use is often limited by acquired drug resistance and undesirable side effects. Much effort has been devoted to the development of new platinum-based drugs which circumvent cross-resistance to cisplatin. We recently found through single DNA observations in solution using fluorescence microscopy that long duplex DNA molecules with a size larger than several tens of kilo base-pairs exhibit a discrete conformational transition from a coil state to a folded compact state upon the addition of various condensing agents, but that short DNA fragments behave like rigid rods and cannot undergo such a folding transition.

In the present, we will show the effect of platinum coordinative compounds on the higher-order structure of a large DNA, T4 phage DNA (166 kbp), by adapting single-molecule observation with fluorescence microscopy. The figure exemplifies the histograms of the long-axis length distributions of T4 DNA molecules together with an assignment of the conformational characteristics of fluorescent DNA images in solution. From the inspection of the time-dependent structural changes, it is concluded that dinuclear Pt(II) complex acts on DNA through both electrostatic interaction and coordination binding.



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P208

1,3-Dipolar Cycloaddition of Nitron Derived from the Unprotected Sugar—A Key Step to 4-Hydroxypipelic Acid Derivatives

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Biological importance, along with variety of synthetic applications makes pipecolic acids the domain of particular interest among other piperidines.^[1] The naturally occurring (2*S*,4*R*)-4-hydroxypipelic acid is a constituent of antibiotics such as virginamicin *S*,^[2] as well as an important intermediate in the synthesis of the HIV protease inhibitor palinavir and NMDA receptor antagonists.^[3,4] The strategy exploiting 1,3-dipolar cycloaddition as a key step reveals advantageous for substituted 4-hydroxypipelic acid.^[5] The crucial byproducts, substituted 1-aza-7-oxabicyclo[2.2.1]heptanes, have been previously obtained in our group in the reaction of nitrones derived from protected sugars.^[6]

Here we report the straightforward 1,3-dipolar cycloaddition of nitron derived from the unprotected sugar as a reasonable possibility for the number of stages reduction in multistep route to substituted 4-hydroxypipelic acids (Scheme 1). The products of 1,3-dipolar cycloaddition were identified as a mixture of two out of four possible diastereomers. Hence the 1-aza-7-oxabicyclo[2.2.1]heptane derivatives were separated by chromatography. The synthesis of optically active 4-hydroxypipelic acid derivatives is then presented on Scheme 2.

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P209

How to Protect Fatal Damage on Genomic DNA: Quantitative Evaluation of Double-Strand Break and Application to Medicinal Chemistry

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There is a growing body of evidence that the oxidative damage to DNA, such as single- and double-strand breaks, cross-links and base modifications caused by various environmental factors, induce mutagenic and carcinogenic processes in living cells. Among these different types of DNA damage, if left unrepaired, double-strand breaks are the most significant, since they can lead to cell death. Numerous in vitro studies have been conducted to detect and characterize DNA strand breaks. The comet assay, or single-cell gel electrophoresis assay, is a rapid and sensitive method for the detection of DNA strand breaks in individual cells. However, intact cells are too complicated to analyze DNA damage in a quantitative manner. It has recently been shown that experimental methodology of single DNA observation by fluorescence microscopy provides the

quantitative information on the degree of double-strand break on genome sized DNAs. In the present paper, we will report how the double-strand damage on genomic DNA is protected through the administration of various biological and chemical agents. The main results are as follows: 1) The double strand damage of genomic DNA molecules become two orders of magnitude less, accompanied by its folding transition onto compact state. Such trend is rather general for the both causes of gamma-ray irradiation and on the reactive oxygens. 2) Protective effect by antioxidants is remarkable against reactive oxygen, whereas it is less effect for the γ -ray irradiation. 3) Probability of double-strand break by gamma-ray linearly decrease with the increase of DNA concentration, when DNA molecules are above several tens kilo base pairs. Such remarkable effect of DNA concentration disappears for oligomeric short DNA molecules.

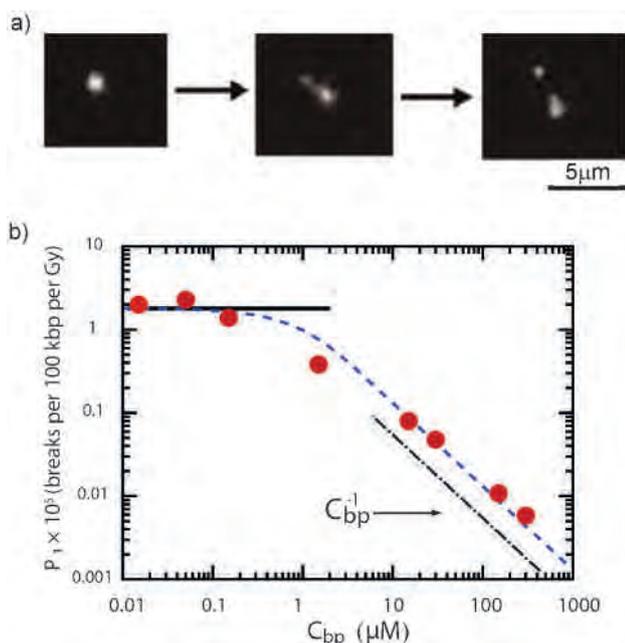


Figure 1. a) Real-time observation on the double-strand break in a genomic DNA (165 kbp) as observed by fluorescence microscope. b) Probability of double-strand break versus DNA concentration deduced from single DNA observation.

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P210

Partial Purification and Characterization of a Lectin from the Coelomic Fluid of the Sea Urchin *Toxopneustes pileolus*

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We attempted to purify lectin from the coelomic fluid of *Toxopneustes pileolus*. Coelomic fluid samples induced hemagglutination in rabbit erythrocytes and the proliferation of mouse spleen cells. The samples were shown to contain glycoproteins by SDS-PAGE. Coelomic fluid was fractionated using a phenyl sepharose CL-4B column to separate coelomic fluid lectins. Of the fractions recovered, PS-I and PS-II were identified as glycoproteins. Hemagglutinating activity was stronger in the PS-I than PS-II fraction. Therefore, the PS-I fraction was fractionated and purified by gel filtration chromatography using a Superdex 200 column. The PS-PI, PS-P II and PS-P III fractions were recovered. The PS-PI fraction showed the strongest hemagglutinating activity among the three fractions and had a heparin-binding property. It showed a nearly single protein band at 960 kDa on native PAGE, which was found to consist of glycoprotein. The PS-PI fraction showed a mitogenic effect on mouse spleen cells from a low concentration. The results of this study suggest that coelomic fluid of *Toxopneustes pileolus* contains physiologically functional lectins.

P211

Ethoxybenzo-thiazole Derivatives as Bifunctional Antihyperglycemic Compounds

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Current pharmacological treatments of type 2 diabetes mellitus (T2DM) include mono- and combination therapies of various orally administered antidiabetic drugs. In many cases these therapies fail to achieve optimal glycemic control. Thus, in view of the epidemic proportions of T2DM and the shortcomings of current antidiabetic

therapy, the need for novel antihyperglycemic drugs is intense. Adenosine monophosphate-activated protein kinase (AMPK) has recently emerged as a major potential target for novel antidiabetic drugs. In skeletal muscles, activated AMPK increases the rate of glucose transport and fatty acid oxidation, while in the liver it predominantly reduces glucose output. These effects lead to increased peripheral glucose disposal and reduced blood glucose levels in hyperglycemic individuals. Various direct and indirect activators of AMPK have been identified. However, side effects, individual intolerance and resistance due to long-term use of such compounds compromise their usefulness and emphasize the need for the development of tissue- and isoform specific AMPK activators. We have recently developed such compounds using an ethoxybenzo-thiazol based pharmacophore model. Several ethoxybenzo-thiazol derivatives have been synthesized and shown biological effects in vitro. The lead compound, 2-((2,3-dihydrobenzo-thiazol-2-yl)methyl)thio)-6-ethoxybenzo-thiazole (EMM-34), increased the rate of glucose uptake concentration- and time-dependently in L6 myotubes nearly 2.5-fold. In addition, his novel derivative augmented glucose-stimulated insulin secretion from the INS-1 beta-cell line. In vivo, it subcutaneous administration lowered blood glucose level in hyperglycemic KKAy mice towards normoglycemic range. Therefore, we use EMM-34 is as a prototype molecule for the development of novel bifunctional antidiabetic drugs that simultaneously increase glucose uptake in skeletal muscles and augment insulin secretion from pancreatic beta-cells.

P212

An Alternative Approach to Drug Discovery: Identification of a Natural Product Privileged Scaffold

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Highly conserved scaffolds embedded in natural products are responsible for their three-dimensional structures. Compounds elaborated from such scaffolds could be used to study the ability to direct functional groups into space in order to interact with their biological targets. 1-Azaspiro[5,5]undecane, a core structure present in different natural products isolated from several plants and marine organisms, has been used as a starting point for a small library of synthetic derivatives. The synthetic strategy used to obtain this scaffold has been optimized to achieve multi-gram scale and can be accomplished in six high yielding steps. The library design has been aimed to assess the scaffold as a privileged or non-privileged molecule and to evaluate its importance as a director of biological interactions.

P213

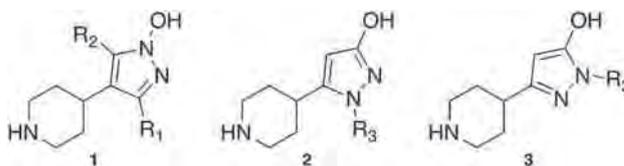
4-(Piperidin-4-yl)-3-hydroxypyrazole: A Novel Scaffold for Probing the Orthosteric GABA_A Receptor Binding Site

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γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system, and the fast synaptic inhibitory transmission of GABA are mediated by activation of postsynaptic GABA_A receptors. Dysfunction in the expression of the GABA_A receptor has been associated with several neurological and psychiatric disorders as epilepsy, anxiety, cognitive deficits, schizophrenia, depression, and substance abuse, making the GABA_A receptors important drug targets for anti-convulsant, anxiolytic, and sedative–hypnotic drugs.

During the last decade the orthosteric binding site has been extensively studied, and numerous important amino acid residues has been identified to be important in the binding of GABA. The combination of these studies and recently reported X-ray structures of the ACh binding protein and nACh receptors has resulted in several hypotheses of the ligand-binding mode, especially the binding of GABA to the receptor. These models, however, is still not reliable enough as GABA is a small and very flexible molecule, which can adopt numerous conformation in the binding site. Therefore, more structural information about the binding mode of ligands to the GABA_A receptor is needed.



In a recent study we have reported a series of 4-(piperidin-4-yl)-1-hydroxypyrazole analogues (4-PHPs, **1**) of the partial agonist 4-PIOL, where several moderate to high potent antagonists were identified ($K_i=5 \mu\text{M}$ to 3 nM).^[1] In the present study we report a new series of analogues of 4-PIOL, based on 4-(piperidin-4-yl)-3-hydroxypyrazole. A series of 1- R_3 -3- (**2**), and 1- R_3 -5-hydroxypyrazoles (**3**) has been synthesised and pharmacological characterized in [³H]-muscimol displacement at native GABA_A receptors and in the FLIPR® Membrane Potential Blue (FMP) assay at the $\alpha_1\beta_2\gamma_2$ GABA_A receptor subtype. All analogues showed affinity to native GABA_A receptors ($K_i=100 \mu\text{M}$ to $0.73 \mu\text{M}$), which indicate a binding mode for the 1- R_3 -3- and 1- R_3 -5-hydroxypyrazoles different from the corresponding 4-(piperidin-4-yl)-1-hydroxypyrazoles.

The present structure–activity studies were rationalized on the basis of a solid homology model of the ligand binding domain of the GABA_A $\alpha_1\beta_2$ dimer. Probable binding modes of the new compounds were proposed and hydrophobic cavities associated to the binding site were identified able to account for the pharmacological data.

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P214

Novel 2-Piperazinyl-3-(arylsulfonyl)quinoxalines as PI3K α Inhibitors

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Phosphoinositide-3-kinases (PI3Ks) are key knots in the PI3K/Akt/mTOR signaling cascade that is closely related to several cellular activities, such as survival, proliferation, growth, apoptosis and motility.^[1] Among the various isoforms identified so far, the α isoform of class I PI3Ks, PI3K α has been extensively studied as a promising target for cancer treatment in recent years. Small-molecule inhibitors of different structures have been reported as PI3K inhibitors with varied potency and selectivity against PI3Ks and related kinases.^[2]

Structural modifications based on series of quinoxalines that were recently reported as PI3K α inhibitors^[3,4] led to a series of novel 2-piperazinyl-3-(arylsulfonyl)quinoxalines, which showed good to excellent antiproliferation activity in low micromolar levels against several human cancer cell lines including PC3, A549, HCT116 and HL60. Enzymatic assay revealed that tested 2-piperazinyl-3-(arylsulfonyl)quinoxaline compounds exhibited micro- to nanomolar inhibitory activity against PI3K α , with the most potent compound WR100 exhibited an IC₅₀ value of 24 nM against PI3K α . Further study showed that these compounds could induce apoptosis in PC3 cell lines. Molecular docking analysis was performed to investigate possible binding mode between target compounds and PI3K. This study indicated the potential of developing 2-piperazinyl-3-(arylsulfonyl)quinoxalines as novel PI3K α inhibitors for cancer treatment.

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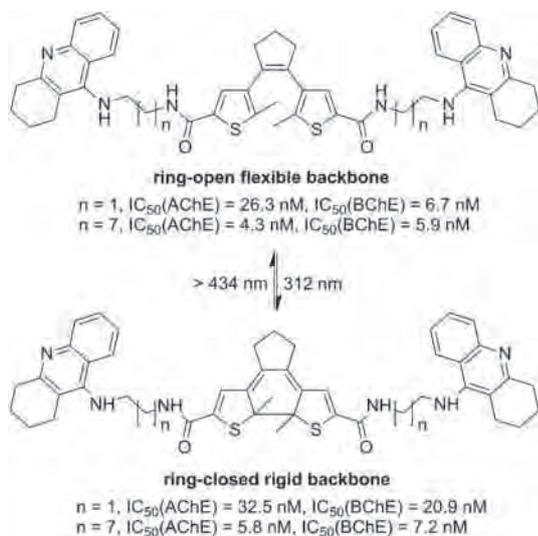
P215

Investigation of Physicochemical and Pharmacological Activities of Photochromic Tacrine Derivatives: Their Inhibition of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) by Both Ring-Open and Ring-Closed Forms

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In order to obtain tacrine-based cholinesterase (ChE) inhibitors with the ability to be 'photo-switchable', photochromic *cis*-1,2- α -dithienylethene-based compounds incorporating either one or two tacrine polyethylenamine derivatives were synthesized and their photochromic as well as biological activities was investigated. Irradiating a methanol solution of all target compounds with 312 nm light resulted in the immediate changes in the UV/Vis absorption spectra, the procedure of which could be reversed by irradiation with visible light ($\lambda > 420$ nm). This ring-closing/-opening cycle could be repeated at least seven times without any sign of degradation. All bivalent compounds show nanomolar inhibition on both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) despite their large and bulky photochromic unit. For the bivalent compound with ethylene connected tacrine ($n=1$), no activity changes were observed at both enzymes after UV irradiation from the colourless ring-open to the violet-red ring-closed form. When applying octylene spacers ($n=7$), again inhibitory activity at AChE was maintained at the nanomolar level, but the mode and mechanism of interaction of the compound with AChE changed when irradiated by light and the ring-closed form inhibited both the catalytic active site (CAS) and also the peripheral anionic site (PAS) of AChE as proved by kinetic studies (substrate-velocity curves and derived Lineweaver–Burk plots). Since interaction with the PAS of AChE can lead to inhibition of its ability to attenuate β -amyloid fibril aggregation, the ability of both photochromic forms to interact with β -amyloid aggregation was investigated. We have obtained compounds with very high and almost identical inhibitory activities in both photochromic forms, in which the mechanism of AChE inhibition can be controlled by UV irradiation. These compounds might serve as valuable molecular tools to investigate the different biological properties of AChE in in vitro assays.



P216

Synthesis and Biological Evaluation of Substituted *N*-Benzylpyrazine-2-carboxamides

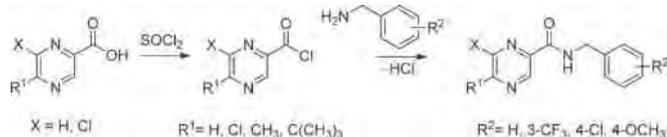
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Worldwide, tuberculosis (TB) is the most common life-threatening infectious disease and remains a major threat to public health. In addition, increased susceptibility to TB in HIV-positive patients is another serious health issue.^[1] Pyrazinamide (PZA), an essential component of short-course antituberculosis chemotherapy, is used as a model compound for substances referred in this research project. Substituted *N*-benzylpyrazine-2-carboxamides were prepared by aminolysis of substituted pyrazinoylchlorides with corresponding benzylamines. Substitution of aromatic ring in benzylamines was based on the experience with analogously substituted *N*-phenylpyrazine-2-carboxamides, which have shown interesting antimycobacterial activity in comparison with PZA.^[2]



Prepared compounds were characterized by analytical data and screened for antimycobacterial (in vitro testing against *Mycobacterium tuberculosis* H37Rv, *M. tuberculosis* I wild stem, *M. kansasii* and two different stems of *M. avium*), antifungal and antibacterial activity.

The compounds were also tested for their photosynthesis-inhibiting activity (PET—the inhibition of photosynthetic electron transport in spinach chloroplasts, *Spinacia oleracea* L.), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, $IC_{50}=1.9 \mu\text{mol L}^{-1}$) was used as a standard.

Several of prepared compounds exhibited relatively good antimycobacterial activity against *M. tuberculosis* H37Rv comparable with PZA, e.g. *N*-(3-trifluoromethylbenzyl)pyrazine-2-carboxamide ($MIC=25 \mu\text{g mL}^{-1}$) or 5-methyl-*N*-(3-trifluoromethylbenzyl)pyrazine-2-carboxamide ($MIC=50 \mu\text{g mL}^{-1}$), which showed the same activity against *M. kansasii* (unsusceptible to PZA). PET—the activity of the studied compounds was moderate or low in comparison with DCMU, e.g. 5-*tert*-butyl-*N*-(3-trifluoromethylbenzyl)pyrazine-2-carboxamide ($IC_{50}=15.6 \mu\text{mol L}^{-1}$).

Acknowledgements: This study was supported by the Grant Agency of the Charles University B-CH/ 710312, by the Ministry of Health of the Czech Republic IGA NZ 13346 and by Grant SVV-2012-265-001.

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P217

Design and Synthesis of Novel Purine-Derived CDK Inhibitors as Antitumor Agents

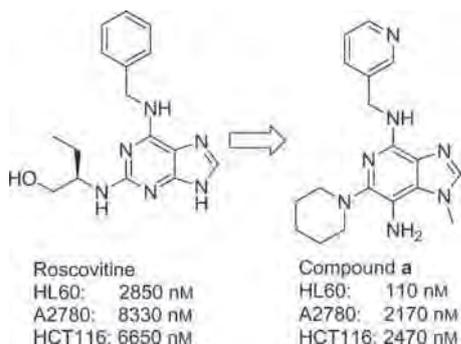
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The therapeutic value of targeting members of the CDK family has been intensively studied and there has been an intensive search for small molecules that target CDKs. During last decades, many CDKs inhibitors have been developed. Moreover, some of them, such as roscovitine, flavopiridol and dinaciclib, have entered the clinical trials as candidate drugs against cancer.^[1,2] Roscovitine, a purine-derived compound, is a pan-selective CDK inhibitor, the further structure optimizations are mainly focus on the substitutions of position 2,6,9 on purine scaffold or replacement of purine scaffold with a bioisostere.^[3–5] While few report the modification on the four nitrogen atoms of purine scaffold.

The co-crystal structure of roscovitine with CDK2 indicates that the nitrogen atom at the position 3 has no direct interaction with any amino acid residue in the ATP binding site of CDK2.^[6] Consequently, we use C-atom to replace the N-atom at the position 3 of purine scaffold. In order to maintain the overall molecular electro-status, electron-withdrawing substitutions were introduced into the scaffold, such as (-CN, -F, -NO₂). Additionally, -NH₂ was also introduced, with the aim of investigating the influence on activity about substitutions with different electronic characters. Herein, we report a series of novel purine-derived compounds were synthesized aimed at enhancing the cellular activity of roscovitine.

Preliminary antiproliferative activity indicated that most of purine derivatives exerted potent to medium cytotoxicity against three tumor cell lines (HL60, A2780 and HCT116). Among them, compound **a** exhibits comparable potency to roscovitine. The CDK2 kinase inhibitory activity and selectivity test is still undergoing.



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P218

A New Class of Dihydropyridines with Neuroprotective Properties

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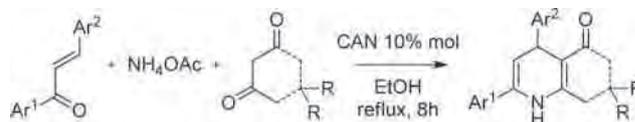
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Oxidative stress increases with the age and is involved in the pathogenesis and evolution of a number of neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis and stroke. The high vulnerability of brain to oxidative damage is related to its high level of oxygen intake, the high content of redox-active transition metal ions and a comparative lack of antioxidant protective mechanisms.^[1] During the last decade, neuroprotection has been increasingly considered as a useful instrument to combat the progression of various neurodegenerative disorders.

Owing to the involvement of a variety of factors in the development of the oxidative damage, varied strategies are being pursued in order to find molecules that could be employed as neuroprotective agents. Calcium dysregulation in the neurons plays a key role in the

molecular mechanism of neurodegenerative disorders by inducing abnormal Ca²⁺ homeostasis, and therefore compounds that are able to regulate the intracellular flow of calcium maintaining it within normal levels may be effective as protecting agents. The main problem with the use of 1,4-dihydropyridine derivatives (DHPs) in the context of preventing neuronal calcium overload is the prevalence of vascular side effects, and for this reason the preparation of neuroprotective DHPs that are designed not to satisfy the well-known structure-activity relationships for vascular activity^[2] is of relevance.

In this communication we present the synthesis and the biological evaluation of a library of 1,4-dihydropyridines and related fused compounds that bear C₆-aryl substituents. Their synthesis was achieved via an efficient three-component process that starts from 1,3-diaryl-2-propen-1-ones, β-dicarbonyl compounds and ammonium acetate, catalyzed by cerium(IV) ammonium nitrate (CAN), acting as a Lewis acid.



We also present their ability to block voltage dependent calcium channels and their neuroprotective effect against Ca²⁺ overload (high potassium model) and oxidative stress in a model of oxygen and glucose deprivation (OGD) carried out in neuroblastoma SH-SY5Y cell line.

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P219

Schistosoma Epigenetics: Targets and New Perspectives

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In this work we present design, synthesis, and in vitro results of some potential inhibitors of histone deacetylase (HDAC) enzyme of the parasitic flatworm *Schistosoma mansoni*, causative agent of a

tropical water-borne illness called schistosomiasis. This disease currently infects 200 million people in 74 endemic countries, and elicit 280,000 deaths yearly, in Sub-Saharan Africa alone.^[1] Schistosomiasis is considered part of the Neglected Tropical Diseases (NTDs) and among human parasitic infections is one of the most widespread in tropical and subtropical areas.

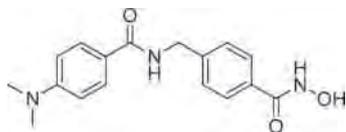
The life-cycle of the parasite needs: a freshwater snail as an “intermediate host”, and humans or rodents as definitive hosts infected through skin contact with contaminated water. The worms live in the venous system, laying eggs that cause massive damage of the liver, bladder, kidneys. Nowadays, there is only one disposable drug: Praziquantel.

Schistosomes change their phenotype more than once during the whole life cycle, which is related to epigenetic regulatory mechanisms. They have an intense metabolic activity and rate of cell division that is outside the control of the host, suggesting an analogy with cancerous growths.^[2]

Starting from our cancer-related experience in the epigenetic field,^[3] we began to investigate possible similarities and differences of the posttranslational machinery between *Schistosomes* and human beings. Up to now, three class I HDACs in the *Schistosoma mansoni* genome (orthologues of mammalian HDACs 1, 3, and 8) have been identified, in addition to three class II HDACs, and five sirtuins.^[4]

Insertions in the SmHDAC8 catalytic domain suggest the potential for disclosure of selective inhibitors for this enzyme, when compared to the mammalian orthologue.

We are currently engaged in design, synthesis and in vitro testing of compounds able to inhibit SmHDAC8. Those inhibitors are tested by fluorescence in vitro assays both on SmHDAC8 and on the human orthologue, in order to determine their activity and selectivity on the targeted enzyme. We are also investigating the anti-schistosomal activity of established HDACi. The activity of those compounds in causing the death of parasites is tested on larvae in culture and apoptosis is detected by TUNEL labelling. Moreover, structural studies in the presence and absence of the HDAC inhibitor SAHA, or with an inhibitor that we have developed, give us the opportunity to better understand the interactions between the SmHDAC8 enzyme and inhibitors at the active site, in order to deduce further optimization strategies for compounds with improved inhibitory activities.



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P220

Applications of Ligand-Based Virtual Screening and De Novo Design in Hit and Lead Structure Identification

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Computer-assisted drug design plays a growing role in early-stage medicinal chemistry programs. Virtual screening is widely employed as a complement to high-throughput screening in hit- and lead discovery to feed medicinal chemists with potential entry points for a specific target.^[1] In addition, de novo design is currently seeing new interest, particularly for the tasks of scaffold hopping, bioisosteric replacement, and even fine tuning of a candidate compound.^[2]

Here, we report some success stories from our group using these computational techniques. We successfully screened a virtual combinatorial library of 1,4-dihydropyrimidines, by use of self-organizing maps. The synthesized molecules showed inhibitory activity against cyclin-dependent kinase 2 (CDK2).^[3]

We also have growing interest in exploring de novo design as a tool to tackle challenging drug targets. Hence, we extensively use our in-house software DOGS^[4] for that purpose. It requires a known bioactive compound as template to grow new molecules in a deterministic and stepwise process. It also places emphasis on the synthesizability of suggested molecules, by proposing synthetic pathways.^[4–6] As an example of the broad applicability of ligand-based de novo design, new constructs representing significant scaffold-hops from amprenavir (HIV protease inhibitor) and VX680 (Aurora A kinase inhibitor), as well as their activities, will be presented. Overall, we show expeditious uses of de novo design, and its potential to become mainstay in hit discovery.

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P221

hLF1-11 Synthesis and Immobilization onto Chitosan Thin Films to Create Antimicrobial Coatings

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Introduction: The human Lactoferrin-derived peptide, hLF1-11, was proven to be highly active against antibiotic-resistant bacteria.^[1] However, the clinical use of this antimicrobial peptide (AMPs) is hampered by the peptide low stability due to fast degradation or to peptide aggregation, as the use of higher peptide concentrations results on higher toxicity levels. AMP immobilization onto a biomaterial surface could be the pathway to overcome these difficulties.^[2] The aim of this work is the development of an antimicrobial surface by covalent immobilization of hLF1-11 onto the surface of chitosan thin films.

Experimental Methods: Chitosan ultrathin films were prepared through the spin-coating of a 0.4% chitosan solution in gold substrates. hLF1-11 immobilization was performed through an SS bound between hLF1-11 terminal cysteine and an *N*-acetyl cysteine previously coupled at chitosan films. Surfaces were characterized using ellipsometry (thickness), Infrared reflection absorption spectroscopy (IRRAS) and X-ray photoelectron spectroscopy (XPS). Bacterial adhesion studies were performed using methicillin-resistant *S. aureus* (ATCC33591). Chitosan films were incubated with this bacterial suspension at 37 °C for 6h and 24h. The viability of the attached bacteria was evaluated using LIVE/DEAD® Bacterial Viability Kit (Baclight™) and fluorescence microscopy.

Conclusions: hLF1-11 peptide was successfully covalently immobilized onto chitosan thin films. Both soluble and attached peptide presented a higher antimicrobial activity than the control chitosan.

Acknowledgements: Thanks are due to the Fundação para a Ciência e a Tecnologia (FCT, Portugal; ref. PTDC/CTM/101484/2008) and to FEDER (ref. FCOMP-01-0124-FEDER-009400) for co-funding the research project.

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P222

Old Drugs with New Faces: Chemical Strategies to Cover Primaquine Unpleasant Traits while Preserving its Attractive Antimalarial Attributes

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Despite the worldwide efforts of Organic and Medicinal Chemists in the arena of malaria chemotherapy since the 1950s, 66-year-old drug primaquine (PQ) is still the only antimalarial in clinical use that is active against all exo-erythrocytic stages of *Plasmodia*, including latent liver forms (hypnozoites) responsible for infection relapse. However, PQ is hemotoxic and presents unfavourable pharmacokinetics.^[1,2] A major factor behind this last aspect is extensive first pass-metabolic inactivation of PQ by oxidative deamination of the drug's aliphatic chain.^[1] This requires frequent administration of high doses of PQ, which brings about serious toxicity issues, as PQ metabolism generates highly reactive oxygen species (ROS) underlying oxidative stress in human cells, namely, red blood cells (RBC). Thus, PQ-based therapy is often associated with hemotoxicity due to abnormal accumulation of methemoglobin in RBC, ultimately leading to hemolytic anemia. This adverse effect is particularly harmful for individuals with deficiency in NADH methemoglobin reductase or in glucose 6-phosphate dehydrogenase (G6PD), the latter being a common trait in African men. Due to this problem, PQ cannot be administered to pregnant women or newborns, as G6PD deficiency cannot be diagnosed in early stages of human life. This is a critical issue in malaria chemotherapy, given that 86% of the fatal malaria cases in 2011 were of children under five years old.

For almost a decade, we have been working on the chemical synthesis and evaluation of peptidomimetic and organometallic derivatives of PQ, designed to be resistant to oxidative deamination while preserving the antimalarial activity of the parent drug; this led to novel PQ derivatives with promising features as drug leads against exo-erythrocytic malaria parasites.^[3–7]

Acknowledgements: This work was supported mainly by the Fundação para a Ciência e a Tecnologia (FCT) (Portugal) and FEDER (European Union), reference nr. PTDC-QUI-65142-2006 and FCOMP-01-0124-FEDER-007418. P.G. and R.M. also thank FCT for financial support to the CIQUP and iMED.UL/CECF research units, respectively.

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P223

Synthesis of Novel 1-Deoxy-sphingoid Bases as Anticancer Agents

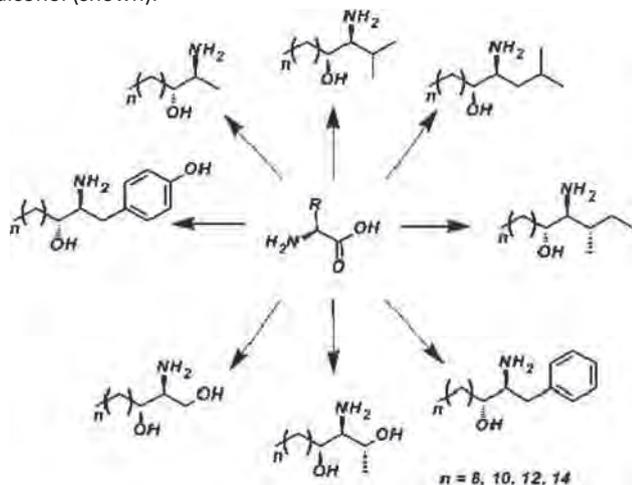
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Sphingolipids (SLs) are essential constituents of eukaryotic cells. SLs are long-chain (which varies from 12 to 30 carbon atoms) aliphatic amino alcohols. This category of amino alcohols is now known to encompass hundreds of compounds that are referred to as sphingoid bases and sphingoid base-like compounds. Most of these compounds participate in cell structure and regulation, and some disrupt normal sphingolipid metabolism and cause plant and animal disease.^[1]

1-Deoxy-sphingoid bases are a different kind of SLs. These compounds have been isolated from some sponges and other sources. They have shown to produce cytotoxic effects as antitumor agents. However, the mechanisms for their biological effects remain unknown. We need more information on structure–activity relationships that can help us to elucidate the biological mechanism of action.

The aim of this work was to synthesize different analogues of (2*S*,3*R*)-2-aminododecan-3-ol. This compound has been isolated from diverse ascidian (*Clavelina oblonga* collected in Brazil^[2] and *Clavelina phlegraea* from the Mediterranean sea) and has shown cytotoxic effect in representative human solid tumour cell lines (A549, lung carcinoma; T-47D, breast carcinoma and AGS, gastric carcinoma).^[3] The synthesis is based in using *S*-amino acids as chiral building blocks and the addition of the appropriate alkyl magnesium bromide to *N,N*-(dibenzylamine)propanal, which is prepared from different amino acids to give the corresponding *beta*-amino alcohol (shown).^[4]



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P225

Molecular Mechanisms of Ligand Binding and Receptor Activation of RF-Amide G-Protein-Coupled Receptors

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Several neuropeptides including the neuropeptide FF (NPFF) and the prolactin releasing peptide (PrRP) exhibit a common carboxy-terminal RF-amide. They have been characterized as ligands for the RF-amide peptide receptor family that belong to the heptahelical G-protein coupled receptors. To date, five subtypes with a great diversity of activities like important neuroendocrine, behavioral, sensory and metabolic functions have been found. Accordingly, the RF-amide peptide receptor family represents a multiligand/multireceptor system, as many ligands are recognized by several GPCR subtypes within one family.^[1]

By peptide synthesis and subsequent testing of the analogues we identified the ligand binding site of NPFF, NPAV and PrRP at their respective receptor.^[2] Furthermore, we used site directed mutagenesis to identify the binding pocket at the different receptors and identified distinct positions that are important for agonist binding. We were able to distinguish between positions, relevant for all receptors, and those, that were receptor subtype and ligand specific. By testing small molecule agonists and antagonists on wildtype and mutant receptors, and subsequent molecular modeling based on recent X-ray structures of GPCRs we could identify the binding mode and suggest a distinct molecular conformation of the receptors for agonist and antagonist binding. By generating the first receptors, that endogenously mimic the ligands, we obtained constitutively active receptors. This nicely confirmed the hypothesis of the activation mechanism and clearly showed for the first time how agonism is obtained and can be introduced by means of chemical biology. Accordingly, the results provide distinct insights into the different binding pockets for RF-amide receptors, which is necessary for the rational development of therapeutic drugs in the NPFF/PrRP system, an important system to target pain, metabolic disorders and cardiovascular diseases.

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P228

Merged Structures as New STAT3 Inhibitors:
The Chimera Compounds

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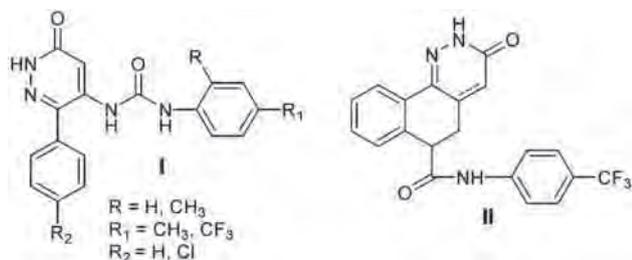
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Signal transducer and activator of transcription 3 (STAT3) is a latent cytoplasmic factor belonging to STAT proteins family. These proteins transduce extracellular signals through the cytoplasm and act as transcription factors in the nucleus, regulating cell growth and survival.^[1] In particular, STAT3 has been found constitutively activated in a broad spectrum of cancer cell lines and human tumors,^[2] and its inhibition specifically suppresses cancer cell survival with only minimal effects in normal cells.^[3,4] In the light of these compelling results, STAT3 represents a promising anticancer drug target,^[5] and we focused our efforts in the discovery of new compounds inhibiting STAT3. During our ongoing researches,^[6,7] we found out several molecules capable of interfering with STAT3 activity. In details, AVS-0288 (a ureidic oxadiazole small molecule, known as an herbicidal agent)^[5] and cryptotanshinone (a natural phenanthrene-quinone derivative)^[8] were identified by a screening performed on a Korean chemical library, whereas DM6 (a new substituted benzocinnolinone) was synthesized in our laboratory. Since these compounds showed an interesting STAT3 inhibitory activity in the dual-luciferase assay, we decided to perform conformational studies and merge their scaffolds with the aim to improve their inhibitory profile. Therefore, starting from these superimpositions, we designed and synthesized the chimera compounds (general formulas **I** and **II**). Their synthesis, crystallographic studies as well as their biological evaluation will be discussed.



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P229

Identification of Small-Molecule Antagonists of
the *Pseudomonas aeruginosa* Transcriptional
Regulator PqsR: Biophysically Guided Hit
Discovery and Optimization

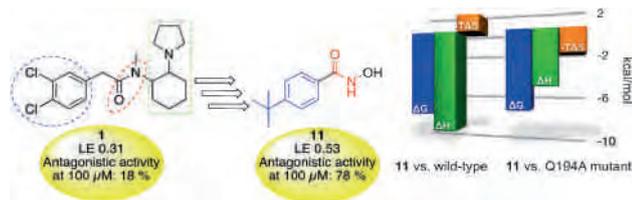
Tobias Klein, Claudia Henn, Johannes C. de Jong, Chris-
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The Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* produces an intercellular alkyl quinolone signaling molecule, the *Pseudomonas* quinolone signal (PQS). The pqs quorum sensing communication system that is characteristic for *P. aeruginosa* regulates the production of virulence factors and biofilm formation.^[1] Therefore, we consider the pqs system as a novel target to limit *P. aeruginosa* pathogenicity without affecting bacterial viability. Recently, we reported on the first antagonists of the transcriptional regulator PqsR, a key player of the pqs system.^[2] However, as their structures are derived from the natural effector HHQ they have insufficient physicochemical properties to be used as a drug.

Here, we present the discovery and optimization of small molecules targeting PqsR. We applied a rational design strategy that involves the simplification of the κ -opioid receptor agonist (\pm)-trans-U50488 (**1**), which was recently found to stimulate the transcription of pqsABCDE in PAO1,^[3] into smaller fragments and analogues. In combination with surface plasmon resonance (SPR) biosensor analysis this approach led to the identification of PqsR binders with good ligand efficiencies (LEs). Determination of thermodynamic binding signatures using isothermal titration calorimetry (ITC) and functional characterization in an *E. coli* reporter gene assay confirmed a promising hit that was elaborated to the potent hydroxamic acid-derived PqsR antagonist **11**. This compound shows a K_D value of 4.1 μM and remarkably it is also potent in *P. aeruginosa*. Beyond this, site-directed mutagenesis together with thermodynamic analysis provided insights into the energetic characteristics of protein-ligand interactions suggesting the presence of hydrogen bonds and CH/ π interactions.

In summary, the rational simplification strategy in combination with biophysical methods, using LE as a primary filter, revealed a promising hit. Future experiments will address hit to lead optimization to open the door for antibiotics with novel modes of action for the treatment of *P. aeruginosa* infections.



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P230

Kaempferol Glycosides from *Olox manni* Leaves: Influence of Sugar Types and Substitution Patterns on Biological Activity

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Olox manni leaves are used in Nigeria for the ethnomedicinal management of cancer and inflammation. Detail chemical investigation of both the ethyl acetate and butanol fractions of the methanol extract of the leave led to the isolation of 16 kaempferol glycosides out of which four are monoglycosides, five diglycosides and seven triglycosides. Rhamnosyl unit was observed in 13 of the compounds, arabinosyl unit in six, glucosyl and xylosyl in two, galactosyl and apiosyl in one. The most common substitution pattern was 3-O and 7-O glycosylation. Of all the isolated compounds, six are new biomolecules not yet reported in the literature: kaempferol-3-O- α -D-apiofuranosyl-(1-2)- α -L-arabinofuranosyl-7-O- α -L-rhamnopyranoside (1), kaempferol-3-O- β -D-glucopyranosyl-(1-2)- α -L-arabinofuranosyl-7-O- α -L-rhamnopyranoside (2), kaempferol-3-O- α -L-arabinofuranosyl-(1-4)- β -D-galactopyranosyl-7-O- α -L-rhamnopyranoside (3), Kaempferol-3-O- α -L-rhamnopyranosyl-7-O- β -D-xylopyranosyl-(1-4)- α -L-rhamnopyranoside (4), kaempferol-3-O- α -L-rhamnopyranosyl-(1-2)- α -L-arabinofuranosyl-7-O- α -L-rhamnopyranoside (5) and kaempferol-3,4'-O- α -L-diarabinofuranoside (6). Also, known compounds 7–16 are isolated and characterized for the first time from the genus *Olox*. Investigation of the biological activities of the isolated compounds, e.g. cytotoxicity, protein kinase inhibition and NF- κ B inhibition, showed that the activities vary in relation to the sugar type, linkages and substitution pattern. This study is a striking case where nature provides rare compounds for SAR studies.

P231

Halogen-Bond-my-Ligand—Generating Hints for Scaffold Decoration

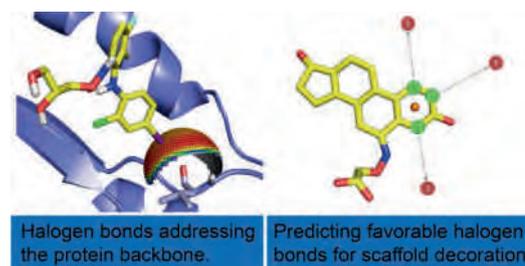
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As a rather new type of noncovalent interaction between ligand and protein, halogen bonding is slowly being integrated into molecular modeling and the drug design process. In principle, aromatic halogenated molecules can form halogen bonds toward any Lewis base.

Based on quantum chemical calculations at the MP2-level, we have evaluated the interaction energies between several halobenzenes and the oxygen of *N*-methylacetamide, representing the carbonyl-function of the protein backbone. In a ligand–protein complex very rarely optimal interaction geometries are observed. In order to assess all spatial dependencies of the halogen bond with regards to deviations from optimal geometries, our calculations include variations in distance, bond angles, as well as in plane and out of plane dihedral angles.

On the basis of these calculations, we developed “Halogen-Bond-my-Ligand”—a tool for scaffold decoration applicable to any crystal structure or docking pose. For every unsubstituted aromatic atom in a ligand the tool determines if halogenation of this position leads to a favorable halogen bonding interaction with the binding site (taking van der Waals clashes into account). In a similar manner, halogen bonding can be integrated into empirical scoring functions for automatic recognition in docking procedures.



P232

Halogen Bonding as a Valuable Interaction Type in Drug Design

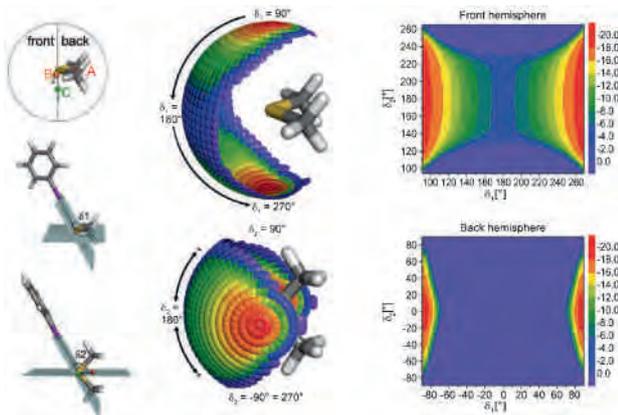
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In the drug discovery process, halogen bonding is a rather new but promising non-classical type of interaction. Compared to other interaction types, halogen bonding is rather directional^[1] and involves an electron donor as binding partner.

Employing quantum chemical calculations on the MP2-level, we explore their applicability in molecular design to address Lewis bases, such as the sulfur atom in methionine^[2] or one of the nitrogen atoms in histidine. Using halobenzenes and suitable model systems for both amino acids, we conducted quantum chemical calculations to investigate the dependency of the interaction energy by the halogen bond geometry. We aim to derive simple interaction rules for medicinal chemists and chemical biologists helping to assess the halogen bonding options in ligand binding sites. Thus, this work aims to facilitate new approaches for halogen bonding-based lead optimization. We suggest that sulfur–halogen and nitrogen–halogen bonds may be useful to expand the patentable medicinal chemistry space.



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P233

Drug Rescue of Mutant p53: Development and Characterisation of Small-Molecule Y220C Stabilisers

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The tumour suppressor protein p53 plays an essential role in the body's defence against cancer. It is inactivated in virtually every cancer either through direct mutation or through perturbation of its associated pathways.^[1] About one third of oncogenic p53 mutations simply destabilise this only marginally stable protein, lowering its melting temperature so that it rapidly unfolds at body temperature.^[2] In theory, wild-type function of these mutants can be recovered by binding of molecules that shift the folding-unfolding equilibrium towards the folded state. We have chosen the cancer hotspot mutant Y220C as a particularly suitable test case for developing and validating such compounds.^[3,4]

Complementing classical fragment screening approaches, we exploited halogen bonding for lead discovery through design and biophysical testing of halogen-enriched fragment libraries.^[5] Subsequent structure-guided design led to potent leads that significantly stabilise p53-Y220C, delaying the aggregation of the mutant protein in vitro and increasing the amount of folded p53 in a homozygous Y220C cancer cell line.

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P234

Halogen-Enriched Fragment Libraries (HEFLibs) Facilitate the Identification of Lead Structures for Rescuing the Mutated Tumour Suppressor p53

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The destabilising p53 cancer mutation Y220C creates a druggable surface crevice.^[1,2] We developed a strategy exploiting halogen bonding for lead discovery to stabilise the mutant with small molecules. We designed halogen-enriched fragment libraries (HEFLibs) as starting points to complement classical approaches.^[3]

From screening of HEFLibs and subsequent structure-guided design, we developed substituted 2-(aminomethyl)-4-ethynyl-6-iodophenols as p53-Y220C stabilisers. Crystal structures of their complexes highlight two key features: (i) a central scaffold with a robust binding mode anchored by halogen bonding of an iodine with a main chain carbonyl and (ii) an acetylene linker, enabling the targeting of an additional subsite in the crevice. The best binders showed induction of apoptosis in a human cancer cell line with homozygous Y220C mutation.

Structure–activity relationships show correlations to QM-based calculations on the interaction energies of halogen bonding. Our structural and biophysical data suggest a more widespread applicability of HEFLibs in lead identification, yielding lead structures that feature binding modes hardly obtainable by other techniques.

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P235

Discovery of a New Class of Gamma Secretase Modulators from a Plant Extract

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Research has shown that long amyloid-beta (Abeta) peptides such as Abeta 42 play a causal role in the development of Alzheimer's disease (AD). Gamma secretase modulators are a promising potential class of AD therapeutics because they reduce the production of these long peptides while allowing the continued production of shorter peptides. Unlike inhibitors of gamma secretase, this biochemical mechanism allows gamma secretase to process other essential substrates such as Notch. By using a screening approach that sought Abeta 42 selective molecules, Satori was able to identify a novel class of gamma secretase modulators. This poster will describe our screening effort and the isolation of our initial hit, SPI-014, from black cohosh (*Actaea racemosa*). It will show that the Satori scaffold exhibits unique pharmacology relative to known gamma secretase modulators by giving different ratios of the shorter Abeta peptide fragments. In addition, early structure–activity relationship studies that laid the foundation for our lead optimization program will be disclosed.

P236

Bodilisant as Highly Useful Fluorescent Histamine H₃ Receptor Antagonists

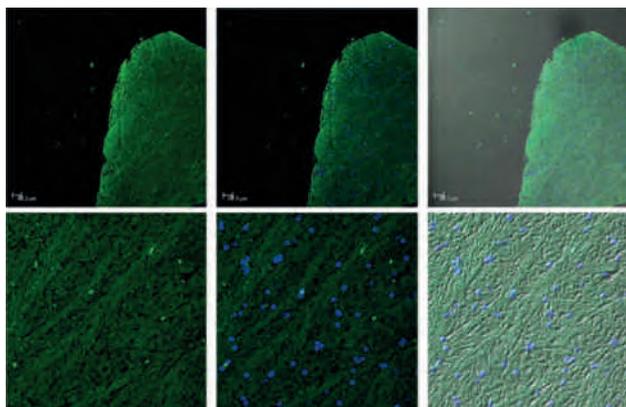
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Different histamine H₃ receptor (H₃R) antagonists have reached late stage in clinical development with still heterogeneous therapeutic indications.^[1] For an improved development of preclinical and clinical candidates novel imaging ligands would be helpful. In enhancement of our previously developed fluorescent H₃R antagonists based on numerous variations of the fluorophore moieties^[2,3] we then focused on novel chalcone derivatives.^[4] In an extension of the physicochemical and pharmacological optimizations, a novel Bodipy dye-labeled compound as a novel fluorescent H₃R antagonist has been developed (named Bodilisant).

Bodilisant showed H₃R affinity in the low nanomolar concentration range with high selectivity vs. the other histamine receptor subtypes tested. With high quantum yield it possesses a wavelength maximum for absorption of 468 nm and for emission of 563 nm. Membrane localization of human H₃R can be visualized in hH₃R over-

expressing HEK-293 cell lines and fully displaced by non-imidazole and imidazole-containing H₃R antagonists. Visualization of hH₃R with Bodilisant in human brain tissue in slices of cerebral cortex and globus pallidus (see figure) has also been successfully applied.



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P237

Lithocholic Acid is an Endogenous Inhibitor of HDM4 and HDM2

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The proteins HDM2 and HDM4 are key negative regulators of the tumor suppressor protein p53, which are frequently upregulated in cancer cells. They inhibit the transactivation activity of p53 by binding separately or in concert to its transactivation domain. HDM2 is also an ubiquitin ligase that leads to the degradation of p53.

We found from in silico screening and confirmed by experiment that the endogenous steroidal bile acid lithocholic acid (LCA) binds to the p53 binding sites of both HDM2 and HDM4 with a 5-fold preference for HDM4. The dissociation constants, in the μM region, are in the range found for binding of LCA to its previously known targets, such as the nuclear farnesoid X receptor. The binding was weakened by structural changes in LCA and so LCA appears to be a natural ligand of HDM2 and HDM4.

LCA induced p53-dependent apoptosis in human cancer cell lines. A closely related structural analog, hyodeoxycholic acid (HDCA) that did not bind to HDM4 in vitro had no effect on caspase activation

in vivo, showing that the apoptotic effects of LCA are p53-pathway specific and not induced by unspecific effects of bile acid treatment. The finding of specific binding of the steroid to HDM2 and HDM4 raises the possibility of new layers of cellular regulation. HDM proteins may be able to act as sensors for specific steroids.

P238

Kinase Profiling by Differential Scanning Fluorimetry

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In recent years, small-molecule kinase inhibitors have emerged to be primary objects of medicinal chemistry research. Due to significant sequential and structural similarity of the ATP-binding site of kinases, developing selective kinase inhibitors still remains a challenging task in drug design.

Differential scanning fluorimetry has been suggested as a technique for small molecular screening.^[1] The environment-dependent change of fluorescence intensity of a fluorescent dye (often SYPRO Orange) is used as a biophysical readout to indicate protein unfolding. Upon binding to the hydrophobic residues of the unfolded protein, which are buried in the core of the protein in the folded state, the fluorescence is typically increased. Ligands occupying a binding site in the folded state should increase the Gibbs free energy of unfolding (ΔG_u) and, accordingly, will shift the apparent melting temperature (T_m) to higher values.

We have demonstrated the use of DSF to measure protein stabilization by pharmacological chaperones.^[2] K_D values derived from concentration-dependent addition of ligand to protein seem comparable to affinities determined by other biophysical techniques. We have shown this in a series of stabilizers of the Y220C cancer mutant of the tumour suppressor p53.^[3]

Herein we apply DSF to kinase screening. Starting from a small selection of kinases involved in cancer and inflammation, we systematically compare different experimental parameters for these proteins. This aims at optimizing assay conditions for a larger panel of kinases with the ultimate goal to use DSF in a standardized way for fast and efficient kinase profiling.

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- [3] *Halogen-Enriched Fragment Libraries as Leads for Drug Rescue of Mutant p53*, R. Wilcken, X. Liu, M. O. Zimmermann, T. J. Rutherford, A. R. Fersht, A. C. Joerger, F. M. Boeckler, *J. Am. Chem. Soc.* **2012**, in press.

P239

Application of DEKOIS 2.0 (Demanding Evaluation Kits for Objective In Silico Screening) for Benchmarking

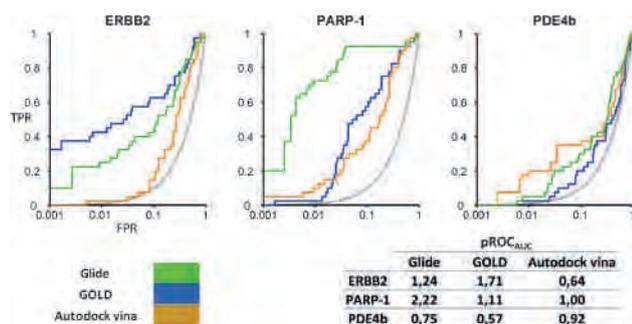
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Benchmark sets are important tools for evaluating scoring functions and docking algorithms especially in the virtual screening and structure-based drug discovery process. Generally designing benchmark sets should meet certain quality criteria to avoid artificial impairment of benchmarking results. Decoy structures should ideally reflect the physicochemical properties of confirmed ligands without possessing structural elements and scaffolds that are responsible for bioactivity at the respective target. To closely achieve this goal in a benchmark design, we herein present an enhanced version and a substantial expansion of publicly available high-quality decoy sets of our previously published, automated and workflow-based (DEKOIS) protocol.

In general, a reliable docking tool should be able to rank actives highest according to their binding scores during the database screening. However, the screening performance of each tool depends strongly on the respective target and—to a certain extent—on the docking parameters. Therefore, benchmarking is a systematic and decisive step for the selection of a suitable docking tool and for the optimization of docking parameters. Thus, we evaluate in this work the screening performance of different newly released docking tools to a wide variety of most cited and targets of high interest to a medicinal chemistry community using our high-quality decoy sets (DEKOIS).

All DEKOIS data sets will be made accessible at www.dekois.com.



P240

Synthesis and Evaluation of Antimicrobial Activity of Novel 2-Aryl-3-benzothiazolyl-1,3-thiazolidin-4-one Derivatives

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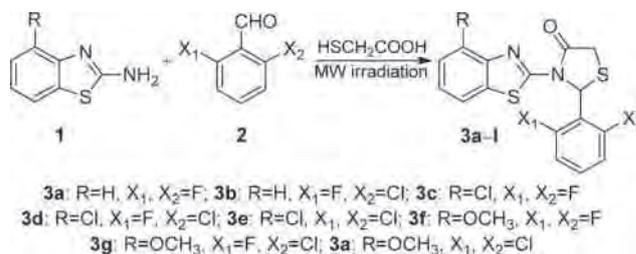
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The increasing resistance to existing antimicrobial treatment has resulted in urgent demand for new classes of antimicrobial agents with different modes of action. For this reason, the current trend in antimicrobial drug design is towards clubbing two or three heterocyclic rings having different sites of interaction. Thus, as part of our ongoing studies in developing new antimicrobials, we report the synthesis of eight novel compounds, incorporating two known bioactive nuclei such as 4-thiazolidinone and benzothiazole.

The synthesis of the title compounds was carried through one-pot three-component reaction between a 4-substituted/nonsubstituted benzo[d]thiazol-2-amine, a 2,6-dihalo-substituted benzaldehyde and mercaptoacetic acid under microwave irradiation (see scheme). All the synthesized compounds were screened for in vitro antibacterial activity against a panel of Gram-positive bacteria (*Listeria monocytogenes*, *Bacillus cereus*, *Micrococcus flavus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Enterobacter cloacae*), using the microdilution method.

The antibacterial activity assay indicated that all synthesized compounds showed moderate to excellent activity against all tested pathogens compared to ampicillin and streptomycin. Furthermore, it was observed that 4-OMe substitution (**3c–e**) enhanced the antibacterial activity, especially against Gram-negative bacteria, while 4-Cl or nonsubstituted compounds were less active.



P241

CoMFA and CoMSIA Studies of 1,2-Dihydropyridine Derivatives as Anticancer Agents

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Taking advantage of our in-house experimental data on 3-cyano-2-imino-1, 2-dihydropyridine and 3-cyano-2-oxo-1,2-dihydropyridine derivatives as inhibitors of the growth of the human HT-29 colon adenocarcinoma tumor cell line, we have successfully developed CoMFA and CoMSIA models. These models yielded highly significant cross-validations (CoMFA: $q^2_{cv}=0.70$; CoMSIA: $q^2_{cv}=0.639$) and excellent predictions of a 5 ligand test set (r^2_{pred} between 0.61 and 0.65). Exploiting this information, synthesis and experimental data directed us to the synthesis of two novel cell growth inhibitory agents with IC_{50} values in the sub-micromolar range.



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P242

New Lipophilic Cinnamic Acid Derivatives. Correlation between Antioxidant and Antiproliferative Activities towards Breast Cancer Cell Lines

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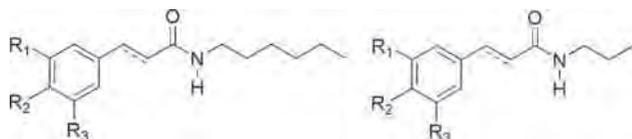
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Dietary phenolic compounds, specifically hydroxycinnamic acids and derivatives, are known to display relevant antioxidant properties as well as biological activity against several tumor cell-lines.^[1] Despite all the interesting biological effects of hydroxycinnamic acids their bioavailability is the major limitation for further clinical applications. Although working well in aqueous media, their hydrophilic nature is usually a restriction to cross membranes,^[2] and to reach intracellular targets. In recent works, we demonstrated that lipophilic derivatives of caffeic and ferulic acids^[3] have increased cytotoxicity against three different human breast cancer cell lines, when compared with the original hydrophilic acids.^[4]

In order to develop new and more effective phenolic agents suitable for chemopreventive and/or chemotherapeutic purposes, amide derivatives of several cinnamic acids (see figure) were designed and synthesized. Subsequently, the compounds were screened in terms of cytotoxicity on two different human breast cancer cell lines, namely MCF-7 and HS578T and on one non-transformed human fibroblast cell line (BJ), which was used as a non-tumor cell control. In addition, the antioxidant activity of the synthesized derivatives was determined in vitro using spectrophotometric assays based on DPPH and ABTS radicals. In addition, the redox potentials have been also determined by electrochemical studies.

From the results obtained, one evident finding is that the original cinnamic acids, in spite of having antioxidant activity, did not inhibit the proliferation of any of the cell lines used. This is probably because its hydrophilicity does not favor the intracellular accumulation of the compounds. Among the lipophilic derivatives, it was possible to observe that compounds with the best antioxidant activity also present higher antiproliferative activity.

In conclusion, one can say that the lipophilic amide derivatives maintain the antioxidant activity when compared to its precursor acids and its increased lipophilicity seems to be crucial for the compounds entering in the cell and exert their cytotoxic effect. Further, there is a positive correlation between the antioxidant activity/redox behavior and the antiproliferative effect meaning that oxidative mechanisms could be involved in proliferation of MCF-7 and HS578T cancer cells.



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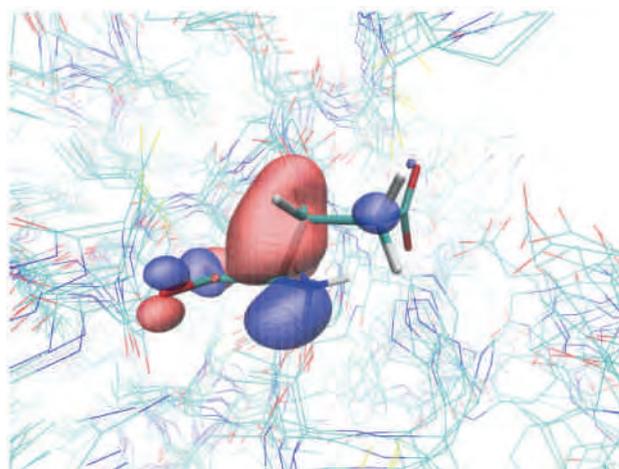
P243

Exploiting Enzyme-Catalytic Power in Virtual Screening and Drug Discovery Using a Transition State Model

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Enzymatic catalysis is possible because of high-affinity binding of an enzyme to the specific geometry and electrostatics of the transition state. This principle was used to design a virtual screening campaign for novel leads against the antibiotic-target glutamate racemase, an enzyme involved in cell wall synthesis. The transition state is the carbanion analogue of glutamate, which was modeled using ab initio methods, and subsequently used to both probe conformations of glutamate racemase that have the highest affinity for the carbanion, and to pre-process the virtual screening library by removing compounds that were geometrically and electrostatically dissimilar to the transition state. Ensemble docking was used to dock the virtual library against relevant conformations of glutamate racemase generated using molecular dynamics simulations in both apo and liganded forms. The compounds were ranked using a consensus scoring scheme, and top scoring compounds were clustered into bins of chemically similar compounds. Representatives from three chemical clusters have been assayed, one of which is a reversible competitive inhibitor with a K_i value of 290 nM against glutamate racemase of *B. anthracis*, and 1.3 μ M against glutamate racemase of *B. subtilis*. The overall approach presented represents a balance between screening based on chemical logic about the enzyme–receptor and sampling in diverse chemical space. This approach has resulted in a significant improvement over direct virtual screening against either the crystal structure or the transition state structure.



P244

Alkaloids from Psychotria as HDAC Inhibitors: A Multifunctional Approach against Neurodegeneration

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Psychotria L. is genus whose neotropical species (subg. *Heteropsychotria*) are characterized by the presence of monoterpene indole alkaloids (MIAs) possessing biological and pharmacological properties on the CNS. MIAs with β -carboline (β Cs) and tetrahydro- β -carboline (TH β Cs) nuclei in *Heteropsychotria* are usually responsible for these biological activities. In the present study, 11 *Psychotria* alkaloids were evaluated for their inhibitory activity on histone deacetylases (HDACs), considered a target for promising disease modifiers in neurodegenerative conditions. These compounds displayed HDAC IC₅₀ values in the μ M range. They are also inhibitors of AChE and MAO-A. Thus, they may be considered as multifunctional candidates for the treatment of neurodegenerative diseases.

P245

Discovery of Muscarinic Acetylcholine Receptor Antagonist and Beta-2 Adrenoceptor Agonist (MABA) Dual Pharmacology Molecules

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Inhaled beta-2 adrenergic receptor (β_2) agonists and inhaled muscarinic acetylcholine antagonists are the most frequently used bronchodilators in the treatment of Chronic Obstructive Pulmonary Disease (COPD). While short-acting agents (4–6h) serve as ‘rescue’ therapy, long-acting (12–24h) bronchodilators can reduce the incidence and number of exacerbations, as well as improve lung function. Due to the complementary nature of the two mechanisms, combinations of the two classes provide even greater improvement in lung function than either mechanism alone.

By applying our multivalent approach to drug discovery, we sought to design muscarinic acetylcholine receptor antagonist and β_2 agonist dual pharmacology bronchodilators. Our initial discovery efforts and early structure–activity relationships including the selection of muscarinic and β_2 pharmacophores as well as the significance of the linker moiety will be described. Several MABA molecules exhibiting bronchoprotection and extended duration of action in our animal models will be highlighted. The proposed multivalent bimodal orientation for these molecules will also be discussed.

P246

Ghrelin Receptor as an Anti-obesity Drug Target: Development of Ghrelin Inverse Agonists and Radiotracers for Imaging

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Ghrelin is a gastrointestinal peptide hormone and is currently the only known orexigenic signal from the periphery. It plays a central role in the short- and long-term regulation of hunger and energy homeostasis.^[1] The ghrelin receptor possesses a naturally high constitutive activity representing 50% of its maximal activity. This basal signaling is thought to induce constant appetite and to trigger food intake between meals.^[2] Consequently, ghrelin antagonists and inverse agonists have emerged as potential anti-obesity drugs.^[3]

The European project GIPIO (gastro-intestinal peptides in obesity) aims to understand hormonal dysfunctions involved in obesity and to design therapeutic peptides/peptidomimetics against this disease. In this context, we developed short peptides possessing a high inverse agonist activity at ghrelin receptor. Modifications such as PEGylation and lipidation were also performed to increase peptides bioavailability.

In addition, ghrelin agonist and inverse agonist radiotracers were developed for PET imaging to give an insight in the hormone behavior and mode of action in vivo.^[4] Information on the pharmacokinetic of ghrelin inverse agonist tracers should also be an asset to develop druggable peptides. With the first in vivo studies, biodistribution and stability of the peptide radiotracers can be reported.

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P247

Antibacterial Properties of Quaternary Chitosan Derivatives

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Quaternary chitosan derivatives varying in their substituent, chain length of the substituent and molecular weight of the chitosan were synthesized (Figure 1) and their antibacterial properties were investigated. Chitosan starting material having five different average molecular weight and degree of acetylation were first subjected to 3,6-di-O-TBDMS protection and N-acylation with chloroacetylchlorides with different chain lengths. Trimethylammonium or pyridinium group was then introduced on the side chain. The activities of these compounds were then tested against clinically important strains of Gram-positive *Staphylococcus aureus* (ATCC 29213) and Gram-negative *Escherichia coli* (ATCC 25922). Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values were determined. Trimethyl chitosan homopolymers with different molecular weight chitosan were also synthesized and used as a reference compound for the investigation of antibacterial activity. In general, the compounds were more active against *S. aureus* with MIC values varying from 4 to 16384 $\mu\text{g}/\text{mL}$ and comparatively less active against *E. coli* with MIC values from 64 to ≥ 32768 $\mu\text{g}/\text{mL}$. The MLC values were the same as the MIC within 1–2 dilutions. In brief, the trimethyl amine derivatives of chitosan polymer were more active than the pyridine derivatives, and their activity increased as the quaternary group (cationic charge) came closer to the polymer backbone, i.e., as the length of the alkyl chain decreased. Thus, synthetic methods to prepare well-defined quaternised chitosan derivatives have been developed and these materials have been used to investigate the structure–activity relationship for the antimicrobial effect.

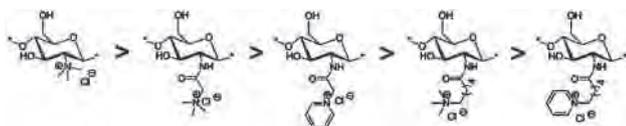


Figure 1. Structures of the quaternary chitosan derivatives synthesized and their order of antibacterial activity.

P248

Building the Survivin/CDK4 Complex by Combining Protein–Protein Docking and Molecular Dynamics Simulations

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Here we describe the structural model of the complex formed by two proteins with important biomedical implication; survivin and the cyclin-dependent kinase 4 (CDK4). Survivin is a type of inhibitor of apoptosis protein (IAP) whose gene is specific and highly expressed in cancer cells. Its association with CDK4 was early demonstrated experimentally but no modeling study helping to understand the molecular details of this protein–protein complex has been published so far, due to the large complexity of this task.

In this work we report the use of advanced modeling methods, including protein–protein docking and GPU-driven molecular dynamics for building the Survivin/CDK4 complex. Additionally, we made use of the surface fractal dimension concept to assess the shape complementarity of the proposed Survivin/CDK4 interface.

The so obtained structural model is highly interesting, since it can be used as starting material for structure-based studies aiming to the design of small molecules acting as protein–protein disruptors with antitumoral properties. In our work we also point out potential positions in the CDK4 surface that can be exploited with this aim, and propose that small molecules that could act as alpha helix mimetics of Survivin are likely to be interesting antitumoral compounds, a hypothesis that waits only to be confirmed by experimental assays.

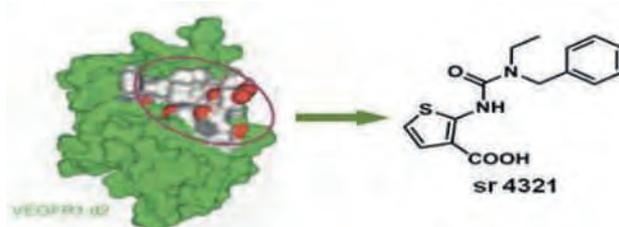
P249

Development of New Antiangiogenic Compounds

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Angiogenesis, defined as the formation of new blood vessels from pre-existing endothelial structure, is a physiological phenomenon but it is also involved in several diseases such as cancer. This process is finely tuned by several regulators among which the vascular endothelial growth factor (VEGF) plays a major role.^[1] Thus, the VEGF/VEGF receptor (VEGFR) system seems to be an important therapeutic target to inhibit tumor growth and metastasis formation.



We develop an antiangiogenic strategy consisting in the design of VEGFR antagonists, disrupting the VEGF/VEGFR complex and the subsequent receptor activation. Based on structural data, we particularly focus on the VEGFR1 ligand. The starting point for the design of nonpeptidic molecules was an in silico screening on the VEGFR1 D2 domain surface. Some small molecules were identified to be well docked on the receptor, and one of the most promising was a (3-carboxy-2-ureido)thiophen derivative, sr4321.

Biological tests on HUVE cells were performed and had showed that sr4321 was able to specifically inhibit the VEGF-induced autophosphorylation of the VEGFR1. It was also demonstrated that this compound has a significant effect on the cell migration and on tubule like formation.^[2]

Today, new studies about this compound are in progress, and in the meanwhile, a structure–activity relationship is realized to synthesize analogues of sr4321.

A synthesis way to quickly access at many molecules and their binding capacity to VEGFR1^[3] will be presented, as well as the cellular tests of the most active compounds.

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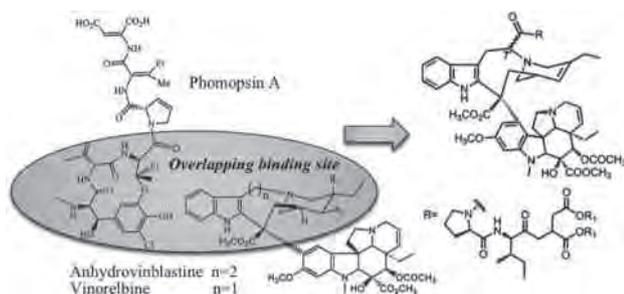
Synthesis and Biological Evaluation of New Vinca Alkaloids and Vinca Alkaloid–Phomopsin Hybrids

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Tubulin plays a key role in many cellular functions such as cell division. Microtubules, resulting from its polymerization, form the mitotic spindle along which chromosomes migrate during mitosis. Tubulin-binding molecules are one of the most important classes of anticancer agents with major drugs already on the market and many promising compounds in clinical trials. Vinca alkaloids^[1] are one of these antimitotic drugs inhibiting tubulin polymerization into microtubules. At present, various vinca alkaloids are commonly used in cancer chemotherapy.

Their precise binding site in the so-called vinca domain was determined in 2005 by Knossow and co-workers,^[2] and it was shown that it partially overlaps with that of the antimitotic cyclopeptide phomopsin A.^[3] The cleavamine moiety of vinblastine and the macrocycle of phomopsin A occupy the same area, however, the vindoline moiety of vinblastine and the lateral chain of phomopsin A are oriented in opposite directions.



These very important results along with those reported previously by our team^[4] prompted us to elaborate a new family of vinca alkaloid and phomopsin hybrids. The interest of this strategy is to obtain original compounds that may interfere with both binding sites leading to an increased activity.

In order to access these hybrids, we first developed a mild and efficient synthesis of new vinca alkaloids with interesting biological activities. Thus, the synthesis and the biological activities of these new vinca alkaloids and the elaboration of vinca alkaloids–phomopsin hybrids will be presented.

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P251

Synthesis and Characterization of *N,N*-Dialkyl Chitosan Homo Polymers and Their Corresponding Quaternary Derivatives

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A highly efficient method for regioselective modification of chitosan biopolymer using a simple reductive amination procedure to yield *N,N*-dialkyl chitosan derivatives was developed. Four different dialkyl derivatives, namely di-methyl, di-ethyl, di-butyl and di-hexyl were synthesized using the 3,6-*O*-di-TBDMS chitosan as a precursor. The reaction involved treatment of the protected chitosan with the aldehyde forming the imine followed by reduction to get the mono alkyl derivative, which was then subjected to similar treatment once again to have the dialkylated product. The use of the TBDMS protected chitosan enabled the reaction to be performed in organic solvent simplifying the method and resulting in 100% substitution. Dialkylation reaction in absence of the protecting groups, i.e., on native chitosan (unmodified chitosan), was performed under acidic conditions, which resulted in lower degree of substitution. *N*-methylation and quaternization of the dialkyl chitosan derivatives was attempted under different reagents and conditions. With the protected dialkyl compound only a very low degree of quaternization was obtained, due to the steric hindrance provided by the highly bulky tertiarybutyldimethylsilyl groups. However, a high degree of quaternization of these derivatives could be achieved by using MeI as reagent and NMP as solvent after the removal of the protecting groups. The quaternization reaction yielded compounds which carried permanent positive charges on the polymer backbone and showed good aqueous solubility, thereby making them suitable for antimicrobial testing. The degree of substitution was calculated from the integrals of ¹H NMR spectrum and all the derivatives were characterized using ¹H NMR, IR and COSY spectrum. These well-defined derivatives will be used for detailed SAR studies of antimicrobial efficacy of quaternary chitosan derivatives.

P252

Synthesis and Biological Studies of Novel Heterocyclic-Bearing Derivatives of Betulin and Betulinic Acid

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Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and structure–activity relationship (SAR) analyses.^[1]

The natural occurring triterpenoids betulin and betulinic acid have been thoroughly investigated during the past years for their anticancer activity.^[2–5] Nevertheless, the poor pharmacokinetic properties of these triterpenoids hampered further pharmaceutical developments.

Several reports have been published demonstrating that either simple or complex modifications may be performed on these lupane-type triterpenoids, without loss of the desired biological properties.^[6,7]

In this communication^[8] we report the synthesis of novel heterocyclic bearing derivatives of betulin and betulinic acid. The *in vitro* cytotoxic activity of the synthesized compounds was evaluated against human hepatocellular carcinoma (HepG2), leukemia (Jurkat), cervical adenocarcinoma (HeLa), colon adenocarcinoma (HT-29), prostate adenocarcinoma (PC-3), and fibroblasts (BJ) cells. The compounds were also screened for their ability to inhibit topoisomerase I.

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P253

2,5-Bis(4-aminophenyl)thiophene Derivatives as Nanomolar-Range Inhibitors of the Botulinum Neurotoxin Serotype A Metalloprotease

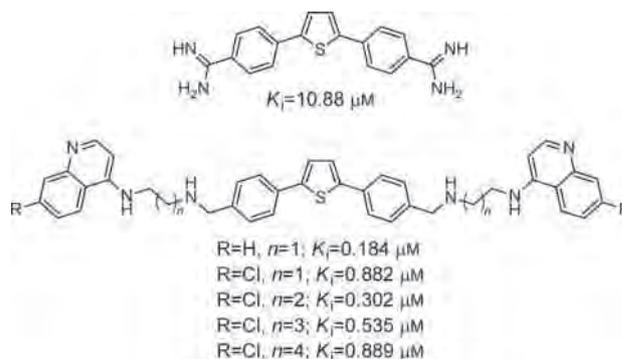
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Botulinum neurotoxins (BoNTs) secreted by *Clostridia* species *botulinum*, *baratii*, and *butyricum* are the most potent of the biological toxins; the lethal dose of BoNT serotype A (BoNT/A) is estimated to be between 1 and 5 ng kg⁻¹ for humans. As a result, these enzymes, which are responsible for the paralysis associated with botulism, are listed as category A (highest priority) bioterror agents by the Centers for Disease Control and Prevention (CDC).^[1] There are seven known BoNT serotypes (identified as A–G).^[2] Each cleaves a component of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which facilitates the transport of acetylcholine into neuromuscular junctions. Di-cationic chemotypes show high potential for BoNT/A LC metalloprotease inhibition, which could be significantly improved with 4-aminoquinolines (ACQ) as substituents.^[3]

Herein, we report the syntheses of new derivatives which are nM-range inhibitors of BoNT/A LC metalloprotease. 2,5-Bis(4-amidinophenyl)thiophene as lead μM-range inhibitor was further developed into new 4-ACQ derivatized 2,5-bisphenylthiophene compounds. Synthesis of the derivatives involved simultaneously replacing the parent inhibitor's terminal bis-amidines with secondary amines and systematic *n*-alkyl-4-amino-7-chloroquinoline substitution. It will be shown strong dependence of introducing of 4-ACQ on inhibition potential of the chemotype.



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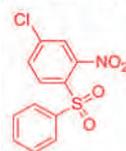
A New Generation of BTB-1 Analogues for Kif18A Inhibition

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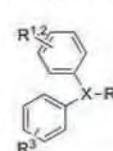
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In cell division the equal partitioning of the genome is mandatory for survival of the organism. Errors in this pathway have severe effects on the viability of the organism and can result in cancer development. The mitotic spindle is a key component for correct chromosome distribution. Various kinesins influence the shape and function of the mitotic spindle. Kinesins are ATP dependent motor proteins, which utilize the energy derived from ATP hydrolysis to produce mechanical force. Kif18A belongs to the kinesin-8 family and is known to be required for the correct alignment of chromosomes at the spindle equator.^[1] Besides its key function in mitosis, Kif18A is characterized by its unique enzymatic properties since it integrates both motility and microtubule depolymerization activity.^[1] In order to understand the function and mechanism of Kif18A, a reverse chemical genetic screen to identify small molecule inhibitors was performed.^[2] The first Kif18A inhibitor, named BTB-1 (**1**), was identified within this screen.^[2] BTB-1 reversibly inhibits the ATPase activity of Kif18A ($IC_{50}=1.69 \mu\text{M}$) in an ATP dependent manner.^[2] In order to establish SARs and to identify the core inhibitory structure, a novel set of BTB-1 analogues was synthesized. The in vitro activity of the compounds was determined by an enzyme coupled assay (ECA). With the derived information, appropriate positions for the attachment of affinity tags were identified in order to elucidate the cellular target. Since Kif18A plays an important role in mitosis and its overexpression was identified in different cancer types^[3,4] small molecule inhibitors could serve as a starting point for novel drug development.

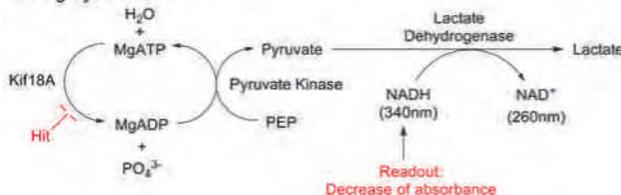
Structure of BTB-1 (1)



Molecular scaffold for analogues



Testing System ECA



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4-Amino-7-chloroquinolines: Inhibitors of Botulinum Neurotoxins (BoNTs) with Antiprotozoal Activity

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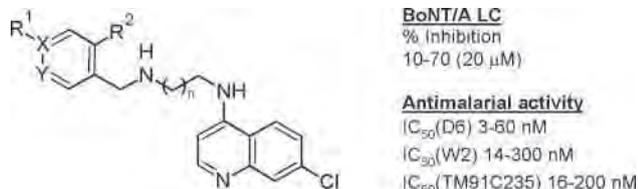
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Naturally occurring botulinum neurotoxins (BoNTs) are the most potent of bacterial toxins. Due to their ease of dissemination and lethality (ca. 1 ng kg⁻¹ for humans) these enzymes are classified as Category A by the US Centers for Disease Control and Prevention (CDC). Consequently, due to the currently limited options for treating BoNT poisoning, there is a significant interest in the development of a small-molecule, non-peptidic, inhibitors (SMNPIs) that would be effective within neurons post-intoxication.

Despite decades of research, malaria is still devastating tropical disease with over 1 million deaths per year. Prevention of malaria is further complicated with the spread of multidrug resistance of many

P. falciparum strains to most of the readily available drugs. Therefore, there is an urgent need for new readily available and safe drugs for prophylaxis and treatment of this disease.

To further investigate the potential of 4-aminoquinoline molecules as multitarget compounds, we synthesized several new derivatives.^[1] Results of biological activity of some synthesized compound indicate that 4-amino-7-chloroquinolines possess the ability to inhibit the three unrelated pathogens: a bacterial toxin (the BoNT/A LC) and protozoan (malaria). Our new results will be discussed.



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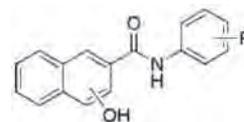
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Synthesis of Hydroxynaphthalene-2-carboxanilides as Biologically Active Compounds

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Infections/diseases caused by invasive pathogens remain a great world problem, thus research efforts are required to develop new compounds as potential antimicrobial agents. The threatening increase of mycobacterial, bacterial and fungal infections occurrence underlines the importance of searching for new antimicrobial chemotherapeutics with targeted effect.^[1,2] The basic strategy of new antimicrobial compounds development is preparation of analogues of clinically used drugs, which would have a better therapeutic index according to SAR study, increased activity against resistant strains, improved bioavailability or a new mechanism of action.



This paper is the result of our interest in ring-substituted naphthalene derivatives and deals with their synthesis and antimicrobial activities. In this study a series of substituted amides of hydroxynaphthalene acid was prepared. The presence of an amide (-NHCO-) group, which simulated a peptide bond, is characteristic of a number of biologically active compounds. These simple structures show wide spectrum of biological effects, such as antimicrobial, antiparasitic, antiviral, antineoplastic, chelating or herbicide activity. Some similar compounds, which show interesting antimicrobial activity, were prepared in recent years.^[3-6] Modification of the anilide part of the molecule resulted in an increase of biological activity.

Acknowledgments: This study was supported by IGA VFU Brno, Project No. 96/2012/FaF.

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Semisynthetic Novel Betulin and Betulinic Acid Derivatives with Improved Antitumor Activity

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Some natural pentacyclic triterpenoids such as betulin and betulinic acid have shown remarkable effects in suppressing tumorigenesis as well as in inhibiting tumor growth.^[1] Anticancer activity of betulinic acid against several types of human cancers including melanoma, neuroblastoma, leukemia, colon, breast, hepatocellular, lung, prostate, renal cell, ovarian and cervix carcinomas, has been reported.^[2-5] Nevertheless, the poor aqueous solubility of lupane triterpenoids, such as betulinic acid, has limited the exploitation of their potential

in both the medical and pharmaceutical areas.^[6] To optimize its pharmacological effects, a number of derivatives of betulinic acid have been prepared and evaluated, mainly targeting on the modification of C-3 hydroxyl, C-20 alkene and C-28 carboxylic acid groups.^[7,8]

In this communication,^[9,10] we describe the synthesis and biological studies of novel lupane imidazole carbamates and *N*-acylimidazole derivatives of betulin and betulinic acid.

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P258

Pyridoacridines Revisited—New, Flexible Approaches to Biologically Active Marine Alkaloids and Analogues

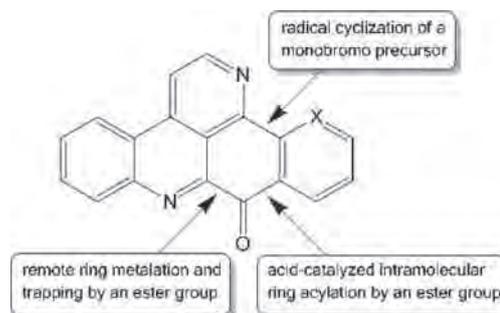
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The pyridoacridines, a group of more than 50 polycyclic alkaloids from tunicates and sponges, exhibit significant biological activities like antitumor, antiviral, as well as antiparasitic activities against *Plasmodium*, *Leishmania* and *Trypanosoma* species.^[1,2]

The most prominent subclass of pyridoacridine alkaloids are pentacyclic compounds derived from ascididemin (X=N), which was isolated from the Okinawan tunicate *Didemnum* sp. in 1988, and shows antitumor and antiparasitic activity. The first total synthesis of this alkaloid was worked out by one of us,^[3] and still represents the most effective approach to the pyridoacridine scaffold. Related marine alkaloids and bioactive analogues (e.g., the antiparasitic desaza analogue; X=CH) differ from ascididemin particularly in the ring A.

This prompted us to develop new approaches to the pyridoacridines which most notably open the opportunity for flexible modifications in the ring A region.



Three independent routes, each going through suitably substituted benzo[*c*][2,7]naphthyridines, and comprising a final cyclization step (indicated by the arrows in the formula), have been worked out. These new methodologies provide a collection of new synthetic analogues for investigation of structure–activity relationships in the pyridoacridine class.

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P259

Development of Small-Molecule Inhibitors of PKA–AKAP Interactions

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A-kinase anchoring proteins (AKAPs) are a family of approximately 50 proteins that directly interact with different receptors, enzymes, ion channels, other signaling molecules and structural proteins in various cellular compartments. In common they have the ability to bind the regulatory subunits (R) of cAMP-dependent protein kinase A (PKA). AKAPs position PKA at defined cellular compartments and thereby coordinate PKA signaling spatially and temporally.^[1] The interactions of AKAPs with R subunits are mediated through an amphipathic α -helix termed R binding domain (RBD), which is conserved within the AKAP family. The RBD binds to a 710 Å² big hydrophobic groove of the dimerization/docking domain (D/D) that is formed through dimerization of the N-terminal parts of R subunits.^[2]

A 25 amino acid long peptide derived from AKAP18 δ , AKAP18 δ -L314E, as well as the 24 amino-acid-long peptide, Ht31, derived from the RBD of AKAP-Lbc bind the RII-dimer with low nM affinity and thereby inhibit globally the interactions between PKA and AKAPs. In renal principal cells, membrane-permeable versions of the peptides inhibit AKAP-dependent, PKA-catalyzed aquaporin-2 (AQP-2) phosphorylation and thereby abolish the arginine-vasopressin (AVP)/cAMP/

PKA-signaling pathway that facilitates water reabsorption from primary urine.^[3] The β -adrenoceptor-induced PKA phosphorylation of different ion channels involved in excitation-contraction coupling in cardiac myocytes is as well PKA/AKAP-interaction dependent and mediates β -adrenoceptor-induced increases in cardiac myocyte contractility. Recently, it was shown that transgenic rat hearts expressing Ht31 respond to isoproterenol (β agonist) stimulation with increased contractility (Langendorf experiments).^[4] We observed a similar effect with a small molecule that disrupts AKAP–PKA interactions and at the same time activates PKA.^[5]

Since the use of peptides and a small molecule with the above described dual effect in animal studies as well as their potential for clinical application is limited, in this work, we used both high-throughput screening (HTS) and in silico design to identify small molecules that specifically bind the RBD-binding pocket of D/D domain and thereby inhibit their interactions with AKAPs.

Three potential disruptors of PKA–AKAP interactions were identified in ELISA-based HTS, among them a pyridinylhydrazone Scaff-004. HSQC-NMR measurements showed that Scaff-004 binds to the D/D domain. Scaff-004 inhibits PKA–AKAP interactions in cell assays and a mouse model in vivo. The structure–activity relationship (SAR) was revealed through synthesizing and testing of >50 analogues. Guided by SAR we developed compounds with 4-fold higher inhibitory potency compared to Scaff-004. Their effects on cardiac myocytes and renal principal cells are currently being tested in cell assays and mouse models.

In addition, we designed in silico a highly functionalized terpyridine as an α -helical mimic of the AKAP18 δ -L314E peptide disruptor of AKAP–PKA interactions based on a concept developed by Hamilton et al.^[6] Several analogues of modeled terpyridine were synthesized. Two of them bind to the D/D domain in HSQC-NMR and ITC experiments. Structurally simplified and synthetically easier accessible analogues are currently being modeled and synthesized in amounts insufficient for biological testing.

In conclusion, we report here small molecules that represent not only novel tools to study compartmentalized AKAP/PKA signaling but, due to their effects in vivo, provide the basis for a new concept in the treatment of diseases associated with aberrations in compartmentalized AKAP/PKA signaling such as cardiac hypertrophy and heart failure.^[1]

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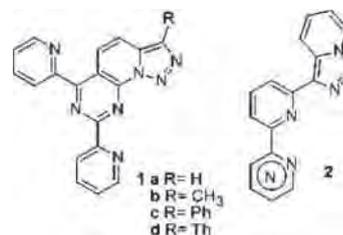
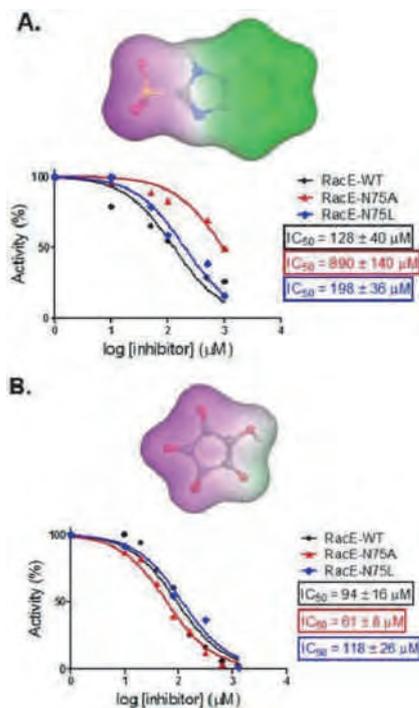
Accounting for Interstitial Waters in Inhibitor Design: Evidence of an Active Role of Water in Glutamate Racemase

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Interstitial waters in enzyme-receptor active sites represent a significant challenge in structure-based drug design. The mutagenesis of active site residue, asparagine 75, in glutamate racemase from *Bacillus subtilis* (RacE) to alanine, or leucine, does not hinder catalytic function, but yields a cryptic effect on inhibitor binding affinities, which is readily explained by examining the role of interstitial water. MD simulations show the N75A mutant enzyme's active site to be joined with a water channel, which is not accessible in the native enzyme or the bulkier N75L mutant enzyme. The nexus of the RacE-N75A active site and water channel results in a concomitant introduction of an interstitial water molecule within the active site. MD studies on the native and mutant enzymes show that the active site-water channel nexus only exists in the N75A enzyme. Importantly, this N75A mutation allows for tight control over the presence or absence of an interstitial water molecule in the experimental system.

In addition to altering the KM of one of the natural substrates (D-glutamate), the interstitial water associated with RacE-N75A has striking effects on inhibitor binding. Two competitive inhibitors, previously characterized by our research group, were assayed against wild-type, RacE-N75A and RacE-N75L. In the case of croconic acid (Figure B), the N75A mutation results in a mild, twofold increase in the inhibitor binding constant (K_i). In the case of 1H-benzimidazole-2-sulfonic acid (Figure A), the mutation results in 26-fold increase in K_i , an approximately +1.97 kcal/mol loss in binding energy. Docking and steepest-descent minimization show this inhibitor molecule competing with the introduced interstitial water molecule for binding to the back of the active site. Thus, the $\Delta\Delta G$ for inhibitor binding is hypothesized to be the energetic cost for ejecting an interstitial water, which is in good agreement with MD studies on the free energy of moving a single water molecule from a favorable interstitial location to bulk solvent (+2.77 kcal/mol; Helms and Wade, *Biophys. J.* **1995**, *69*, 810). These results imply an active role of water in ligand binding where interstitial waters, if correctly considered, can substantially dampen or enhance the potency of inhibitors.



DNA binding tests have been done studying variations in fluorescence emission and UV absorption of the compounds, when DNA is added. Spectrophotometric titrations have also been done using specific DNA (poly(dA-dT)₂ and poly(dG-dC)₂), to determine interaction specificity. DNA viscosimetry titrations were also performed, revealing that compounds **1** interact with DNA in a non-intercalative way. Finally, compounds **1** have been tested as antileishmanial agents, obtaining good results against four species of *Leishmania* (*L. infantum*, *L. braziliensis*, *L. guyanensis* and *L. amazonensis* promastigotes) for **1b** and **1c**.

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[1,2,3]Triazolo[1,5-a]pyridines and Their Copper(II) Complexes. Studies of DNA Interactions

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DNA, as carrier of genetic information, is a major target for drug interaction due to the ability to interfere with important biological processes governed by this biomolecule.^[1] The main DNA interacting compounds are either polycyclic, aromatic and planar ligands,^[2] or Ru, Pt or Cu complexes of this kind of ligands.^[3] In our synthetic work focused on generating new derivatives from [1,2,3]triazolo[1,5-a]pyridine nucleus, we have synthesized two series of compounds with general structures **1**,^[4] and **2**. These molecules and their Cu²⁺ complexes have been evaluated as possible DNA binders.

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Discovery of a New Small-Molecule Inhibitor of p53–MDM2 Interaction

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The p53 tumour suppressor is a major regulator of cell proliferation and death. In tumours that retain a wild-type (wt) p53, the activity of this protein can be inhibited by the endogenous negative regulator MDM2. In this case, inhibitors of p53–MDM2 interaction have been

considered promising drugs for cancer therapy.^[1,2] Developing small molecules that modulate protein–protein interactions is difficult, owing to issues that include the typical flatness of the interface, the difficulty of distinguishing real from artefactual binding, and the size and character of typical small-molecule libraries.

Yeast assays consisting of *Saccharomyces cerevisiae* cells co-expressing human wt p53 and MDM2 have been used for the screening of small-molecule inhibitors of p53–MDM2 interaction. In these assays, inhibitors of p53–MDM2 interaction, such as Nutlin-3A, revert the inhibitory effect of MDM2 on p53-induced growth inhibition/cell cycle arrest as well as on p53-dependent transcriptional activity of a reporter gene (described in [3]). Using this approach, a chemical library of small molecules synthesised by the CEQUIMED-UP group was tested and the small molecule LEM1 was identified as inhibitor of p53–MDM2 interaction.

The identified compound (LEM1), with favourable apparent permeability coefficient and no cytotoxicity on normal human cell lines, exhibited promising activities as inhibitor of p53–MDM2 interaction in two human tumour cell lines derived from breast cancer (MCF7) and colon carcinoma (HCT116 p53+/+). The results obtained confirmed that 10 μ M LEM1 treatment stimulated p53-dependent transcriptional activity, led to p53 protein stabilization, increased p21 and Bax protein levels, and induced caspase-7 activation in human tumour cell lines. Notably, these effects were not observed in the HCT116 p53-/- derivative cell line.

Though the molecular mechanism of action of this compound was validated in human tumour cell lines, the molecular interaction site of LEM1 is still unknown. In order to evaluate the molecular basis of disruption of p53–MDM2 interaction by LEM1, X-ray crystallographic studies will be carried out by checking possible molecular interactions between LEM1 and MDM2. For that, several attempts to obtain a recombinant human MDM2 (amino acid residues 17–125) expressed in *Escherichia coli* BL21 (DE3) RIL, using the pEX-N-His expressing vector, were performed. This MDM2 fragment was purified by affinity chromatography, using Ni-NTA agarose column.

The higher simplicity of the synthetic process of LEM1 when compared with that of other inhibitors of p53–MDM2 interaction (e.g., Nutlin 3A) will certainly guarantee economic advantages for the commercialization of this compound as an additional research tool in the p53 field. Additionally, LEM1 represents a promising small molecule to be further explored as anticancer drug and/or as a lead compound toward the synthesis of more potent and selective inhibitors of p53–MDM2 interaction.

Acknowledgements: This work was supported by the FCT (Fundação para a Ciência e a Tecnologia) through REQUIMTE (PEst-C/EQB/LA0006/2011) and CEQUIMED-UP (Pest-OE/SAU/UI4040/2011), FEDER funds through the COMPETE program under the projects FCOMP-01-0124-FEDER-015752 and FCOMP-01-0124-FEDER-011057, and by U. Porto/Santander Totta, and in part by the Italian Association for Cancer Research, AIRC (IG #9086). A.M. Paiva thanks for a grant (PTDC/SAU-FCT/100930/2008) in the scope of the project. M. Leão (SFRH/BD/64184/2009) and J. Soares (SFRH/BD/78971/2011) are recipients of FCT fellowships.

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Exploring the Orthosteric nACh Receptor Binding Site by Conformational Restriction of the nACh Agonist DMABC

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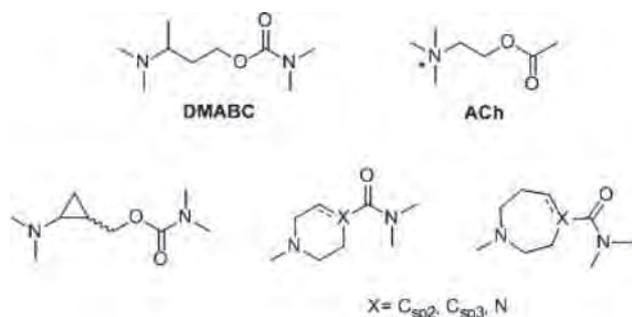
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If we are to talk about classic approaches in Medicinal Chemistry conformational restriction and controlled geometry of ligands is a must. Old but never outdated, this approach is being used for a better understanding of the topography and interactions that occurs at the orthosteric binding site of nicotinic acetylcholine receptors (nAChR). The orthosteric binding site of these ligand-gated ion channels is located in the interface of α - β or α - α subunits. The amino acid sequences that form this binding site are highly conserved among the different receptor subtypes, therefore, achieving a high degree of selectivity turns out to be a challenge that requires a fine tuning in the design of potential selective ligands in order to exploit the small differences found in the receptor cavity.

DMABC is a small synthetic agonist related to acetylcholine (ACh) and exhibits a significant selectivity towards the $\alpha_4\beta_2$ subtype. The predicted linear binding conformation of this molecule, similar to that of ACh or epibatidine, was shown to be in disagreement with a recent X-ray crystallography study, which revealed a folded conformation of DMABC to ACh-binding protein. Based on these new findings, three series of DMABC analogues, cyclopropane, piperazine/piperidine and azepine/azepane containing derivatives, were designed, synthesized and pharmacologically characterized in a [³H]-epibatidine binding assay at the $\alpha_4\beta_2$, $\alpha_3\beta_2$ and $\alpha_4\beta_4$ subtypes and a FLIPR membrane potential blue assay at the $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes.

The synthesized compounds represent different degrees of conformational restriction of DMABC, and in general the results reveal strict structural requirements regarding stereochemistry and conformation for activating the nAChR.



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Study of the Molecular Mechanism Governing Functional Selectivity at the Serotonin 5-HT_{2A} Receptor

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The phenomenon of functional selectivity explains how different ligands, acting on the same G-protein coupled receptor, can differentially activate diverse intracellular pathways.^[1] The relevance of functional selectivity in the treatment of schizophrenia is suggested by experimental observations of biased agonism at the serotonin 5-HT_{2A} receptor, an important target for antipsychotic therapy. It has been established that different ligands acting on this receptor can activate to different degrees phospholipase C and phospholipase A₂ pathways.^[2] These results suggest that a detailed knowledge of the molecular mechanisms governing this phenomenon would permit a finer understanding of the effects of antipsychotic drugs on cellular signaling and would allow obtaining more effective and safer drugs.

In the present work, we present preliminary results of a study oriented to unveil the molecular mechanisms guiding ligand-induced functional selectivity. In a first step, we have built structural models of complexes between the 5-HT_{2A} receptor and series of biased ligands associated with different functional selectivity profiles in order to identify key ligand–receptor interactions discriminating compounds with different behaviors. In a second step, the characteristics of these complexes have been further analyzed by conducting molecular dynamics simulations in order to identify more subtle differences related to changes in the equilibrium between different GPCR active conformations.^[3]

In this communication, we will show some examples of the obtained complexes and review some preliminary hypotheses on the key ligand–receptor interactions that lead to stabilization of different receptor conformations linked to the activation of a certain pathway.

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P267

Residence and Recognition Time and Their Use for Structure–Kinetic Relationship Studies and for Judging Efficacy of Lead Series

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Binding strength, affinity (K_D), measured by surface plasmon resonance (SPR) biosensors, can be broken down into kinetic rate constants that reflect drug–target recognition (the association rate constant, k_{on}) and the stability of the resulting complex (dissociation rate constant, k_{off} or residence time, $1/k_{off}$). The relationship between these properties can be described as $K_D = k_{off}/k_{on}$. In a lead series, k_{on} and k_{off} can vary by several orders of magnitude even when compounds have similar affinities. It is very important, therefore, to measure and understand the binding kinetic pattern of lead series and the effect this might have on the PK/PD properties^[1–3] of the scaffolds. Structure–activity relationship (SAR) studies^[4,5] can be improved by plotting the structures of the lead series in an $k_{on}/k_{off}/K_D$ graph to display structure–kinetic relationships (SKR) as a 2D map for true interpretation on how a change in structure really influence the drug–target binding.

The effects of different on/off-rate combinations in relation to the bioavailable concentration of leads and how this influences binding to the target protein and the residence time of the drug in the binding site will be discussed, along with several SKR examples.

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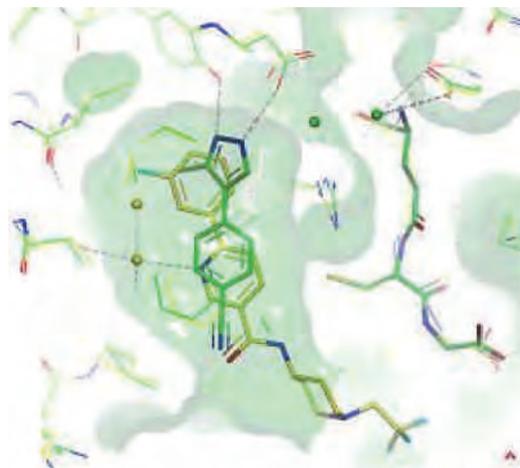
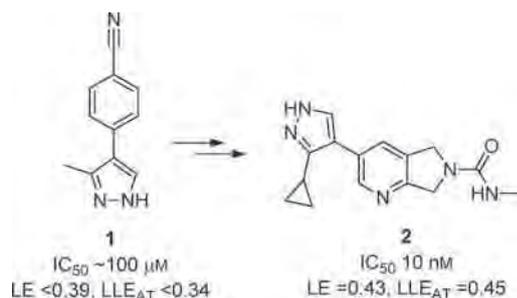
P268

Fragment-Based Drug Discovery Applied to Identify Soluble HPGD2 Synthase Inhibitors

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Solubility and pharmaceutical properties are important factors in drug attrition and development complexity. Here, fragment-based drug discovery and rigorous adherence to lipophilic ligand efficiency^[1] were specifically targeted to develop molecules with excellent physicochemical properties such that DCS^[2] class 1 properties were the goal from early in the discovery programme. Thus, using fragment-based screening techniques, 4-(3-methyl-1H-pyrazol-4-yl)-benzotrile (1, ~100 μM) was identified as a novel low molecular inhibitor of hematopoietic prostaglandin D2 synthase (PDGS). The inhibitor evokes a novel protein movement in what is thought to be a lipophilic pocket; however, the protein liganded structure shows novel polar interactions.^[3] Subsequent fragment optimisation followed by fragment growing afforded a lead molecule (2) with good solubility and evidence of oral activity in a pharmacodynamic model.



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Discovery of a New Class of iGluR Antagonists comprising the Quinoxaline-2,3-dione Scaffold as Distal Carboxylic Acid Bioisoster

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In the central nervous system (CNS), (S)-glutamate (Glu) functions as the major excitatory neurotransmitter. Once released from the pre-synaptic neuron into the glutamatergic synapse, Glu activates a number of pre- and post-synaptic glutamate receptors. On the basis of the pharmacological profile and ligand selectivity studies, the Glu receptors have been divided into two main classes: ionotropic receptors (iGluRs) and metabotropic receptors (mGluRs). Moreover, the iGluRs have been divided into three groups on the basis of ligand selectivity studies: AMPA, KA (kainate) and NMDA receptors.

Those receptors are believed to be involved in many neurophysiological processes, thus, psychiatric diseases or disorders such as depression, anxiety, addiction, migraine, and schizophrenia may be directly related to disordered glutamatergic neurotransmission.^[1] A better understanding of the different receptor subtypes is essential and may aid the development of new drugs.

One strategy by which the independent role and function of a receptor subtype can be studied, is by use of subtype selective ligands. Thus, the design and synthesis of new lead structure which might lead to novel subtype selective ligands is of great interest.

Previous works has shown that substituted quinoxaline-2,3-diones such as DNQX acts as α -amino acid bioisoster and are antagonists at AMPA and KA receptors.^[2] Here is presented the discovery of a

new class of iGluRs antagonists where the quinoxaline-2,3-dione moiety is introduced on the side chain of an α -amino acid and thus functions as a carboxylic acid bioisoster. Two structures with varying length of the amino acid side chain were designed on the basis of modeling studies, successfully synthesized and shown to be ligands at the iGluRs.

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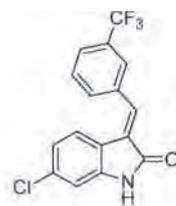
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Functionalized Benzylidene Indolinones: Synthesis and Antiproliferative Activity on Hepatocellular Carcinoma Cell Lines

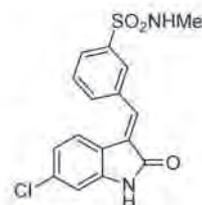
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Hepatocellular carcinoma (HCC) is an aggressive malignancy with poor prognosis. It is strongly associated with hepatitis B and C infections and is prevalent in developing countries which bear 84% of the global disease burden.^[1] There is only one FDA-approved drug (sorafenib) for HCC. In our investigations for novel agents for HCC, 6-chloro-3-(3-trifluoromethyl-benzylidene)-1,3-dihydroindol-2-one (**47**) was identified in our laboratory to have potent and selective effects on the viability of hepatocellular carcinoma (HCC) cells. In an attempt to further improve the activity and selectivity profile of **47**, additional derivatives of **47** were synthesized and evaluated for their growth-inhibitory potency on a HCC cell line, HuH7.^[2] Compounds with comparable potency to **47** were further evaluated on additional hepatocellular carcinoma cells as well as non-malignant cells, IMR 90 and TAMH. In this way, two compounds **1-18** and **6-6** were identified as promising alternatives to **47** with sub-micromolar potencies on HCC cell lines and selective growth inhibitory effects on HepG2 and HuH7 as compared to non-malignant cells. Both analogues together with **47** were further investigated for CYP1A1 induction, effects on alpha fetoprotein (AFP) transcription in HuH7 and phosphorylated signal transducer and activator of transcription 3 (p-STAT 3) levels in HepG2. We found that **1-18** and **6-6** had limited effects on the CYP1A1 activity. **6-6** reduced transcription of AFP in HuH7 cells, similar to **47**, but this was not observed for **1-18**. Neither **47** nor **6-6** reduced levels of p-STAT3 at 50 μ M in contrast to **1-18**, which reduced p-STAT3 at 30 μ M after 2 h incubation with HepG2 cells. Taken together, **1-18** and **6-6** are promising leads for further development as novel anti-HCC agents, with Stat-3 signaling as a potential target of **1-18**.

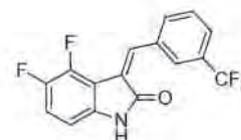


47
 IC_{50} : $0.46 \pm 0.06 \mu$ M (HuH7)
 IC_{50} : $9.23 \pm 0.88 \mu$ M (IMR 90)
 Solubility (pH 7.4): $0.03\text{--}0.04 \mu$ g mL⁻¹
 Cl_{op}: 5.08



1-18

IC_{50} : $0.54 \pm 0.06 \mu$ M (HuH7)
 IC_{50} : $2.10 \pm 0.35 \mu$ M (IMR 90)
 Solubility (pH 7.4): $6.65\text{--}11.91 \mu$ g mL⁻¹
 Cl_{op}: 2.97



6-6

IC_{50} : $0.54 \pm 0.07 \mu$ M (HuH7)
 IC_{50} : $8.10 \pm 0.73 \mu$ M (IMR 90)
 Cl_{op}: 4.68

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P271

[1,2,3]Triazolo[1,5-*a*]pyridine Derivatives as Potential Chemotherapeutic Targets for Leishmaniasis and Trypanosomiasis

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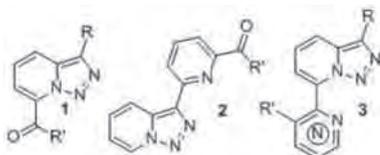
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Leishmaniasis caused by the kinetoplastid parasite *Leishmania spp.* is among the most important parasitic diseases, affecting millions of people and considered to be within the most relevant group of neglected tropical diseases.^[1] American trypanosomiasis, caused by the flagellate *Trypanosoma cruzi*, is an insidious, potentially fatal

parasitic disease that is widespread in Latin America affecting 10–14 million of people or more. Also in the United States, Canada, Spain, Italy, Israel and Australia causes concern due to the high rate of immigration existing.^[2]

Therefore, chemotherapy is the main approach to control these worldwide spread diseases. Several compounds of great pharmaceutical interest contain the triazole ring, various 1,2,3-triazole derivatives are reported in literature to be effective against different protozoos species.^[3] In order to find new drugs with antileishmanial or trypanocidal activity, we have synthesized and evaluated eleven 1,2,3-triazolopyridyl ketones (**1**), two 1,2,3-triazolopyridyl ketones (**2**) and nineteen 1,2,3-triazolopyridyl azines (**3**).

Compounds **1**, **2**, **3** were screened against *L. infantum*, *L. braziliensis*, *L. guyanensis* and *L. amazonensis* promastigotes. In addition, the efficacy of the most active compounds was also studied towards the intracellular amastigote forms. Compounds **1** and **2** were screened against *T. cruzi* epimastigote and trypomastigote. In order to determine the selectivity index, the cytotoxic properties were evaluated against J774, and RAW macrophages.



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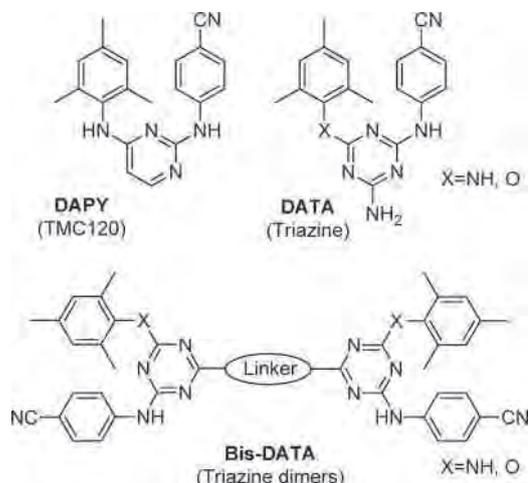
Synthesis and Antiviral Activity of Novel Triazine Dimers

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HIV-1 reverse transcriptase (RT) is one of the most popular targets in the field of antiretroviral drug development. Etravirine (Intelence™, TMC125), a diarylpyrimidine (DAPY, second-generation NNRTI) was approved in 2008 for the treatment of HIV-1 infection in treatment-experienced patients who show evidence of HIV-1 resistant strains. Another DAPY compound rilpivirine (Edurant™, TMC278) was ap-

proved in 2011 as a single product and in combination with emtricitabine and tenofovir (Complera™) for untreated HIV patients. Dapivirine (TMC120), another DAPY compound is currently under development for HIV-microbicidal applications. The excellent pharmacological profiles of the DAPYs have encouraged several research groups to explore next-generation NNRTI agents for the treatment and prevention of HIV infections.



In the present study, we wish to report our efforts in the discovery of novel compounds based on a triazine core through a bivalent ligand approach in which two triazine moieties are covalently connected by suitable linkers (**Bis-DATA**). All the compounds were evaluated for their anti-HIV-1 activity (wild-type) and cytotoxicity in TZM-bl cells in comparison with **TMC120** and **DATA**. Furthermore, selected compounds were tested against single and double mutant strains. In addition, enzyme inhibitory assays were performed with selected compounds against HIV-1 wtRT.

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Discovery of Novel Anti-inflammatory Drug Candidates Designed as p38 MAPK Inhibitors

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The causes of inflammatory and neuropathic pain are fundamentally different. However, there are common mechanisms underlying the generation of each pain state, including the activation of

mitogen-activated protein kinases (MAPKs). Tissue injury is associated with inflammation and induces inflammatory pain. In particular, p38 mitogen-activated protein kinase (p38 MAPK) is activated by inflammatory mediators in primary sensory and secondary order dorsal horn neurons and participates in the generation and maintenance of inflammatory pain. An important condition characterized by inflammatory pain is the rheumatoid arthritis, a chronic systemic inflammatory disorder of autoimmune origin.

New drug development efforts for the treatment of inflammation and related disorders targeting p38 MAPK have been led and several p38 MAPK inhibitors have been developed, as exemplified by the urea derivative BIRB-796, which concluded Phase II of human clinical trials for rheumatoid arthritis.

Recently, we described the anti-inflammatory profile of compound LASSBio-998, designed as p38 MAPK inhibitor.^[1] However, despite the good anti-inflammatory profile, this compound has pharmacokinetic limitations that indicated the need for structural optimization.

In this abstract, we describe the design, synthesis and pharmacological evaluation of new urea-derivatives analogues of LASSBio-998. These urea derivatives were designed by docking studies, using the p38 MAPK structure obtained from the Protein Data Bank (PDB ID: 1KV2, at 2.8 Å resolution); and were synthesized in good yields.

Considering that TNF- α is a cytokine implicated as causal agent in the onset and progression of inflammation, and it is regulated by p38 MAPK signaling pathway, we have investigated the ability of these urea derivatives to inhibit TNF- α and IL-1 β production in cell line THP-1 cultures. These compounds inhibited these cytokines production and were also able to inhibit p38 MAPK phosphorylation evaluated by immunoblot experiments.

The analgesic and anti-inflammatory activities of LASSBio-998 (300 $\mu\text{mol/kg}$) and its ureidic analogues (LASSBio-1494, 1495, 1496, 1497; 100 $\mu\text{mol/kg}$, p.o) were evaluated employing a model of antigen (mBSA)-induced arthritis in mice. All derivatives (1 h pre-treatment, p.o) caused a marked reversion in the mechanical hypernociceptive threshold and also inhibited cell migration to the joint cavity.

The post-treatment with LASSBio-1495 (100 $\mu\text{mol/kg}$, p.o.) also reduced the nociceptive response induced by mBSA injection up to the 4th hour after treatment showing the therapeutic applicability of this compound in an established inflammatory/pain state.

Simultaneously, studies of chemical, plasma and microsomal stability were performed. These urea derivatives were stable at pH 7.4 and pH 2.0, and presented plasma and microsomal half-lives ranging from minutes to hours. Compounds LASSBio-1494 and LASSBio-1495 showed the longest half-lives.

Enzymatic assays employing the activated p38 α MAPK are also in progress to determine the ability of these urea analogues to directly inhibit this protein kinase by quantification of substrate phosphorylation.

In summary, we have discovered novel potential p38 MAPK signaling pathway inhibitors, orally active, stable at pH 7.4 and pH 2.0, and with adequate plasma and microsomal half-lives, standing out compound LASSBio-1495. This urea derivative showed relevant anti-inflammatory and analgesic activities in chronic models of pain and inflammation, indicating its promising potential in the oral treatment of rheumatoid arthritis.^[2]

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Synergic Strategies against Bacterial Multidrug Resistance

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Multidrug resistant (MDR) bacteria, informally known as “superbugs”, are the results of decades of use and misuse of antibacterial agents, which triggered the development of resistance to all antibiotics in clinical use independently from the biological target of the drug.^[1] One promising strategy to contrast this phenomenon is the synergy between on-field clinical research, in silico, and wet-lab chemistry. This approach allows to timely respond to the rise of resistance through a series of steps including: i) clinical isolation, ii) genomic characterization, iii) resistance mechanism identification, iv) new target definition, v) drug optimization.

In this communication, preliminary results from a recent project on drug development against MDR bacteria are reported.^[2,3] These include the setup and validation of a chemoinformatic strategy based on a molecular interaction field (MIF). Modeling studies included a recently developed algorithm called Fingerprints for Ligands and Proteins (FLAP)^[4] and were performed to evaluate possible differences, with respect to Linezolid, in the interaction of the most active compounds in the series with the 50S ribosomal subunit. The validation of this approach has been achieved through the synthesis and bioactivity testing of two series of Linezolid-like 1,2,4-oxadiazoles.

Acknowledgements: Financial support from the Italian MIUR within the “FIRB-Futuro in Ricerca 2008” Program, Project RBF08A9V1, is gratefully acknowledged.

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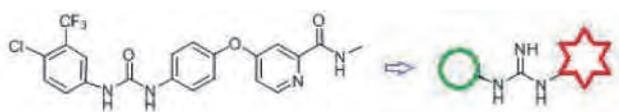
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P275

Towards New Inhibitors of Protein Kinases: Guanidine Analogues of Sorafenib

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In the last five years, we have developed different families of guanidinium-like derivatives that are DNA minor groove binders (MGBs) and some of them can also induce apoptosis in promyelocytic leukaemia HL-60, neuroblastoma Kelly and breast carcinoma MCF-7 cells. These MGBs exhibit some structural similarities with Sorafenib (see figure), which is used for the treatment of kidney and liver carcinomas. This drug is the first oral multikinase inhibitor that targets serine/threonine and receptor tyrosine kinases in both tumour cell and vasculature. These kinases play a key role in the regulation of cellular proliferation and death and thus, Sorafenib has a dual action to stop the growth of cancer cells: (i) inducing apoptosis by targeting the Raf/Mek/Erk^[1] pathway and (ii) inhibiting tumour angiogenesis by targeting receptors such as VEGFR 2, 3 and PDGFR.

Considering the mentioned similarities between our compounds and Sorafenib, and in a 'rational' multitarget approach for the treatment of cancer, we have prepared four different families of guanidinium-based analogues of Sorafenib. During the synthesis of these molecules, a novel simultaneous reduction of nitro and carbonyl groups was discovered.^[2]

Different biochemical assays have been performed over the final derivatives. Hence, viability assays and different kinases inhibition tests have been performed to assess their cytotoxicity and to evaluate their role as potential protein kinase inhibitors. In the viability experiments, a number of these compounds from families I, II and III were found to inhibit HL-60 cell viability in the low μM range, similar to Sorafenib. Inhibition assays performed on CK-1 δ and GSK-3 kinases gave negative results for all families of compounds. However, in the RAF-1/MEK-1 kinase pathway, compounds from family III display 86–99% inhibition. Furthermore, one of these compounds from family III shows 20% anti-angiogenesis activity by inhibiting the VEGFR at 10 μM . The inhibitory effect of all of these compounds was also tested on ERK-1/2 and p-38 MAPK and only moderate inhibition (20%) of both kinases was found.

We now plan to test their ability to induce apoptosis of a number of cancer cell lines. Computational docking studies will be executed to analyse the binding interaction of the compounds with the different targets in order to rationalise our biological results and allow for improvements in the anticancer activity of future compounds.

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Do the Target Proteins of a Promiscuous Ligand Have Similar Binding Sites?

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Background: Selectivity is a key factor in drug development. Predicting rapidly and at low cost the binding of a molecule to several thousands of targets is now possible using computing approaches to chemogenomics. Among the structure-based methods, the comparison of protein binding sites benefits from the ever growing information in the Protein Databank and from recent technical developments.^[1] In this context, we studied the characteristics common to the three-dimensional structures of complexes between a promiscuous ligand and its different target proteins.

Methods: The sc-PDB database^[2] was parsed to extract all ligands in complex with multiple targets. Ligands clearly identified as highly flexible or poorly druggable, such as monosaccharides, nucleotides or fatty acids, were not investigated. All targets of a promiscuous drug-like ligand were systematically compared at the sequence and structural levels. In practice, we analyzed 1070 proteins pairs for a total of 247 different ligands, using the program CE for fold comparison^[3] and three in house programs for site comparison (Fuzcav, Shaper and SiteAlign).^[4,5]

Results: As expected, we observed that promiscuous ligands generally bind to similar sites even though global fold or sequence is not conserved between the two compared proteins. Nevertheless, we could not evidence a structural similarity between the binding sites for about 25% of the proteins pairs. We further examined the ligand flexibility, the binding mode, the crystallographic water and the affinity issues, thereby identifying subtle relationships between ligand–protein recognition and binding site similarity.

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P277**Novel Ursolic Acid Derivatives with Improved Antitumor Activity**

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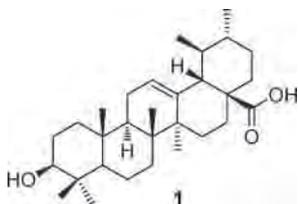
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Cancer is the fourth leading cause of death worldwide, with a tendency to increase in the next years due to the increase in life expectancy.^[1] Natural products provide a promising source for developing effective anticancer agents.^[2] Ursolic acid (**1**) is a pentacyclic triterpenoid present in plants, vegetables and fruits, and has been found to have several biological activities including antitumor activity.^[3] Several groups have done structural modifications of ursolic acid (**1**) aiming to increase its antitumor activity.

In order to improve the pre-existing antitumor activity of ursolic acid (**1**), a panel of semisynthetic derivatives were prepared by introducing heterocyclic rings into several points of the backbone structure of ursolic acid (**1**). The antiproliferative activities of these new derivatives were evaluated in several cancer cell lines including pancreatic, prostate and breast cancers. These compounds have improved antiproliferative effects in those cancer cell lines with arrest of the cell cycle in the G1 phase and induction of apoptosis.



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P278**New Ursolic Acid Derivatives with Potent Antitumor Activity in Pancreatic Cancer Cells**

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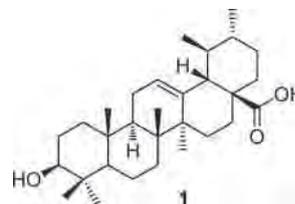
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Pancreatic cancer is the fourth of cancer-related death with a survival rate of six months.^[1] Natural products are a great source of developing anticancer agents, and approximately 50% of current used chemotherapeutic drugs are derived from natural products.^[2] Ursolic acid (**1**) is a pentacyclic triterpenoid present in fruits and vegetables and has been found to have some antitumor and chemopreventive activities in pancreatic cancer models.^[3]

Fluorine is a highly desirable atom, since its introduction into the key positions of molecules could improve metabolic and chemical stability, membrane permeability and binding affinity.^[4] Fluorine has been added into the currently used anticancer agents. Using ursolic acid (**1**) as starting material, several fluoro-derivatives were prepared and evaluated for their antiproliferative activity in pancreatic cancer cell lines with improved antiproliferative activity and act through novel mechanisms. These novel fluoro-derivatives have potential to be developed as therapeutic agents for pancreatic cancer treatment.



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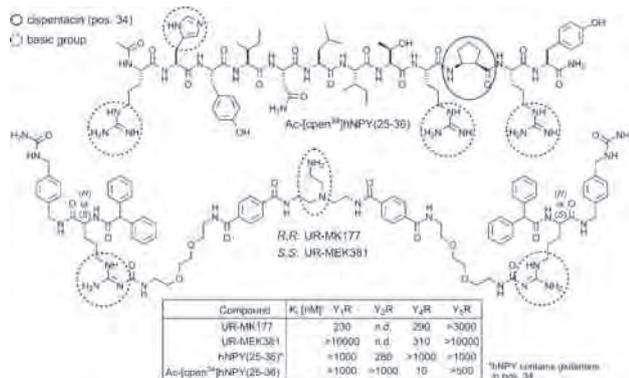
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Bivalent Argininamide-Type Neuropeptide Y₁R Ligands as Lead Structure for the Development of Nonpeptide Y₄R Antagonists

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Peptides of the neuropeptide Y (NPY) family (NPY, PYY, PP) are involved in the regulation of numerous physiological processes, such as blood pressure, food intake, pain sensitivity, anxiety/anxiolysis and hormone release. In humans, these peptides address four functionally expressed NPY receptor subtypes (Y₁R, Y₂R, Y₄R, Y₅R), which belong to the superfamily of G-protein-coupled receptors. Potent and selective nonpeptidic antagonists were described for the Y₁, Y₂ and Y₅ receptors, but are still lacking for the Y₄R, the only NPY receptor subtype preferring pancreatic polypeptide (PP). Recently, we designed and prepared a series of bivalent Y₁R ligands linking two entities of the (*R*)-argininamides BIBP 3226 or BIBO 3304, both highly potent and selective Y₁R antagonists. Interestingly, the bivalent ligands showed poor Y₁R selectivity, in particular over the subtypes Y₂ and Y₄. Some of them even proved to be equipotent at the Y₁R and Y₄R, for instance compound UR-MK177 with binding constants (K_i) of 230 and 290 nM, respectively (see figure). Its enantiomer, the (*S,S*)-configured bivalent argininamide derivative UR-MEK381, was prepared and proved to be inactive at the Y₁R, but still exhibited high Y₄R binding (K_i=310 nM) and antagonistic activity (K_b=140 nM). Thus it is the most potent nonpeptide Y₄R antagonist known to date. UR-MEK381 shows structural analogies to the 12-amino-acid C-terminal fragment of NPY (NPY(25–36)). Lately we could show that the introduction of artificial amino acids, such as cispentacin, into NPY(25–36) has great impact on the NPY receptor subtype preference pattern, even leading to potent and selective partial agonists for the Y₄R (e.g., Ac-[cpen³⁴]hNPY(25–36); see figure). In combination with structure–activity data of such peptide ligands, compounds like UR-MEK381 are considered an important structural basis for the development of highly potent and selective nonpeptide Y₄R antagonists.



P280

2-Arylpauillons: Synthesis and In Vitro Antitrypanosomal Activities

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Tropical diseases like leishmaniasis and trypanosomiasis cause high mortality and morbidity in tropical and subtropical regions worldwide. In the absence of safe, effective and affordable treatments against these neglected diseases, the development of new drugs is needed.^[1–3] Since both leishmaniasis and trypanosomiasis are caused by unicellular parasites belonging to the *Trypanosomatidae* family, they may display similar biological drug targets and could be susceptible to similar drug chemotypes.

In a recent paper, paullone chalcone hybrid structures were reported as antileishmanial agents.^[4] When representatives of this compound class were evaluated in vitro against bloodstream forms of African trypanosomes (*Trypanosoma brucei rhodesiense*), a considerable antiparasitic activity was identified. Paullone chalcone hybrids contain a Michael acceptor substructure, which may give rise to unwanted toxic side effects. In order to remove this undesirable element, it was replaced by aromatic ring systems. The so-designed 2-arylpauillons retained antitrypanosomal activity and showed an acceptable toxicity profile on a THP-1 monocyte cell line and NIH 3T3 fibroblasts.

The presentation will display two synthetic routes towards 2-arylpauillons. A key step in the synthetic route leading to 2-phenylpauillons was a Suzuki–Miyaura cross-coupling reaction of 9-*tert*-butyl-2-iodopaullone^[4] with substituted phenylboronic acids. A second series of congeners comprises 2-hetarylpauillons, which were obtained by cyclocondensation of the chalcone paullone hybrid structures with suitable nitrogen binucleophiles.

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In vitro antidermatophytic activities of the derivatives against *Microsporum gypseum*, *Trichophyton mentagrophytes* var. *erinacei*, and *Epidermophyton floccosum* were screened by the broth micro-dilution method. Terbinafine, itraconazole, ketokonazole, flukonazole, and griseofulvin were used as control agents.^[8] Cytotoxicity was evaluated by the maximum nontoxic concentrations (MNTCs) of each sample, which were determined by the method described previously^[9] based on cellular morphologic alteration. All of the compounds exhibited significant antidermatophytic activities; in particular, halogen-bearing derivatives were found to be more active than chlorokojic acid against *Epidermophyton floccosum*.

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P283

Capture Compound Mass Spectrometry and Molecular Modeling: Proteomics and Computational Chemistry Tools in the Detection of New Protein–Ligand Interactions

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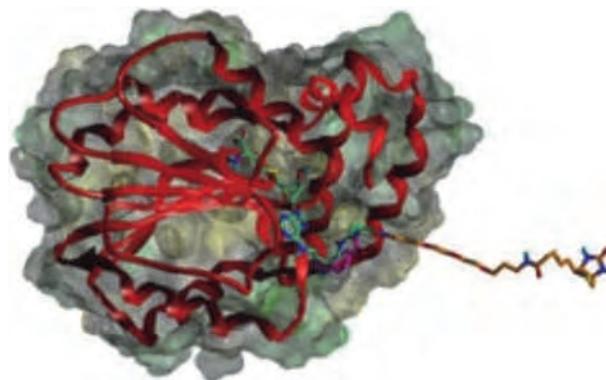
Assessment of protein binding profiles for small molecules has become a crucial issue in different fields of research. Extensive target identification for drugs or drug candidates gives insight into the mode of action, but also unravels new binding partners for an already-known structure, opening the door to new indications for the drug or drug candidate. Also, proteins correlated to toxic side effects such as hepatotoxicity can be detected. Such side effects are a major cause for failure of drugs in clinical trials, and the interactions between drug and off-targets underlying this toxicity are difficult to assess.

Capture Compound Mass Spectrometry (CCMS) has served successfully in this field. Capture Compounds™ are trifunctional probes: a selectivity function (a small molecule) interacts with the target

protein(s) in a biological sample, a reactivity function irreversibly forms a covalent bond, and a sorting function allows the captured protein(s) to be isolated for mass spectrometric analysis. The formation of a covalent bond between the Capture Compounds and the protein(s) of interest allows the isolation even of weakly interacting or low abundant proteins. Determination of the crosslink positions of the Capture Compound can be used for detection of the selectivity function / small-molecule binding site on the isolated proteins.

Molecular modeling complements this process. Investigations of Capture Compound binding to protein structures or homology models gives insight into the crosslink process and is used in the design of Capture Compounds for specific purposes, and ligand–protein complexes for the novel targets can be modeled based on the experimental Capture results.

In close collaboration of our Proteomics and Medicinal and Computational Chemistry facilities, Capture Compounds for difficult targets could be designed, the binding of the small-molecule probes to new targets could be elucidated, and the SAR of compound series explained. This combined approach gives clear directions for the design of bioactive compounds or drugs on a very early stage of compound design projects.



P284

Competition-Based Screening and Characterization of Fragments with SPR AND ITC

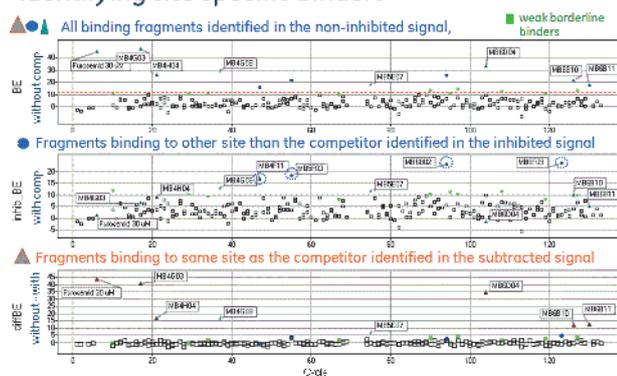
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Today, the binding of thousands of fragments to drug targets can be screened using surface plasmon resonance (SPR) biosensors. The screening can also be performed in competition mode, and the binding fragments can be directly binned into groups with: 1) known binding site, 2) allosteric site(s), and 3) nonspecific binding. During characterization binders are validated by isothermal titration calorimetry (ITC), and both detailed binding site specificity and thermodynamic properties of the fragment–target interaction can be extracted. In this poster we compare five competitive assay designs, three using SPR and two using ITC: 1) use of immobilized

low-molecular-weight site definition compound for analysis of target–fragment binding in solution, 2) direct binding of fragments to blocked and unblocked target, 3) binding of fragments to partly blocked target, 4) ITC competition titration experiments, and 5) single-injection ITC competition experiments. We present results from a case study in which SPR-based competition screening was initially used for selection of binding site specific and allosteric hits and for elimination of false positives. The fragment hits were then characterized by a combination of SPR and ITC in the different competition modes. Binding kinetics and thermodynamics were also used in the characterization of the fragments. The pros and cons of the different competition methods are discussed.

Identifying site-specific binders



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Focused Pseudo-static Hydrazone Libraries Screened by MS Binding Assays—Optimizing Affinities towards GAT1

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MS binding assays have been described as a powerful tool in the search of new drug candidates.^[1,2] Their feasibility for the screening of compound libraries generated by means of dynamic combinatorial chemistry was demonstrated by developing pseudo-static hydrazone libraries targeting GAT1, the most abundant GABA transporter in the CNS. Due to the fact that dynamic combinatorial libraries often suffer from unequal concentrations of test compounds, monodimensional libraries were generated by completely converting four equally concentrated aldehydes with an excess of a single nipecotic acid derived hydrazone. A competitive MS binding assay, employing a native MS marker, then reveals the binding quality of a library. Former investigations disclosed the hydrazone derived from biphenyl-2-carbaldehyde as promising hit with an affinity of pK_i 6.186 ± 0.028 . The present study focused on the biphenyl system for further optimization of the binding affinity. Pseudo-static focused hydrazone libraries were generated by combining diversely substituted biphenyl-2-carbaldehydes

with the hydrazone derivative in excess. Subsequent deconvolution showed that lipophilic groups at the 2'- and 4'-positions could significantly increase the affinity. The most potent library component was found to exhibit a pK_i value of 8.094 ± 0.098 , surpassing the potency of the biphenyl derivative by almost two log units.

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P286

Use of Advanced Knowledge-Management Tools for Maintaining an Updated Repository of Toxicologically Relevant Information

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Open PHACTS^[1] is an IMI^[2]-funded project aiming to build a platform (OPS) that will provide access to the vast amount of open-access biomedical resources present on the internet.^[3,4] The OPS will make use of semantic web^[5] technologies that allow flexible and fully integrated access to all available information from a single entry point without the need to align and integrate the data from a myriad of heterogeneous data sources.

In the present communication we describe a use case application of the OPS for extracting data that will be used for building and updating toxicity predictive models, developed in the context of eTOX,^[6,7] another IMI project. This application illustrates the potential of this technology for solving practical problems common in drug development.

In this work, we used the OPS platform and framework to build a data-crawling application which will automatically distil, from multiple public sources, a series of compounds annotated against relevant toxicological endpoints. This information is automatically curated using multi-step protocols and used to keep an updated local repository in a format suitable for building predictive models. In this communication we compare the results of this data extraction tool with information extracted from other open sources and discuss the pros and cons of the use of automated tools like the one described here as well as its potential applications in other fields.

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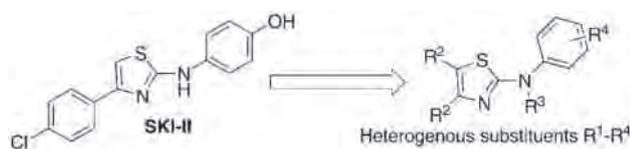
P287

**Selective Sphingosine Kinase Inhibitors—
Pharmacological Tools to Study
Immunomodulatory and Proliferative Processes**

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Sphingolipids exert key roles in the regulation of fundamental (patho) physiological processes. The pathogenesis of cancer and autoimmunity can be directly linked to sphingolipid metabolism. Sphingosine and sphingosine-1-phosphate (S1P) have been identified as counterplayers for maintaining homeostasis of cell functions. Imbalance toward anti-apoptotic S1P leads to hyperproliferation and inflammation.^[1,2] An important therapeutic strategy to combat elevated S1P levels are sphingosine kinase inhibitors. The two isoenzymes of sphingosine kinase (SphK1, SphK2) are responsible for S1P formation. Both differ in many aspects: amino acid sequence, subcellular localization, tissue distribution, substrate specificity, and binding partners. Even contrary effects on cell fate have been described, with SphK1 acting anti-apoptotic and SphK2 acting pro-apoptotic. Rational drug design of selective SphK inhibitors therefore concentrates on optimization of the non-selective SphK inhibitor SKI-II by the combination of structural motifs known from already described SphK inhibitors and novel modifications.^[3]



We screened our inhibitors pharmacologically by two in vitro assay systems. Initial selection was performed at fixed doses (percentage SphK1/2 inhibition) by using a fluorescence-based ADP-detecting assay. Affinity was measured by microscale thermophoresis (i.e., measurement of the Soret effect as a result of varied molecular motion across a temperature gradient) with fluorescently labeled SphKs. Screening of our compound library revealed initial promising candidates for further in vivo testing. These potential pharmacological tools are a promising approach to test the importance of sphingosine kinase regulation in health and disease.

Acknowledgements: Supported by Else Kröner-Fresenius-Stiftung and LOEWE OSF, LIFF, NeFF, Anwendungsorientierte Arzneimittelforschung (Fraunhofer IME), and EU COST Actions CM1103, BM1107, and BM0806.

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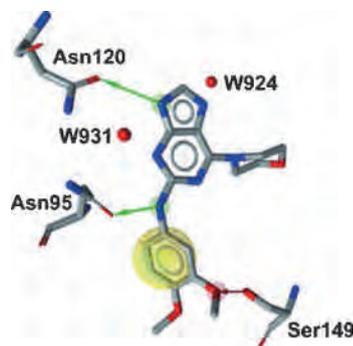
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**In Silico Discovery of Novel Purine-Based DNA
Topoisomerase II α Inhibitors as Potential
Anticancer Agents**

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DNA topoisomerases are an important family of enzymes that catalyze the induction of topological changes (e.g., relaxation/supercoiling, catenation/decatenation, and knotting/unknottng) of DNA. These enzymes perform their function by creating transient double-stranded breaks in the DNA molecule. Given their role in topological changes, topoisomerases represent key targets for the development of novel anticancer agents.^[1] Human topoisomerase II α is a homodimer that consists of three domains and bears close homology with its bacterial counterpart, DNA gyrase.^[2]



Topoisomerase II targeting agents generally fall into two large groups that differ in their mechanism of action. The first group—poisons—stabilize the covalent cleavable complex and convert this enzyme into a cellular toxin that is lethal to normal cells. The second group includes catalytic inhibitors that act at different stages of the catalytic cycle.^[3,4]

The aim of our research was to identify novel inhibitors that act by interfering with the catalytic cycle of human topoisomerase II α by blocking ATP binding. Based on the available structural information for topoisomerase II α ,^[5] an in silico virtual screening campaign was designed combining molecular docking calculations with three-dimensional structure-based pharmacophore models. A novel class

of purine-based inhibitors with micromolar activity was discovered. The binding of these compounds was subsequently investigated extensively by a powerful surface plasmon resonance (SPR) technique.

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Molecular Modeling of IKK2 Target: Structural and Ligand Binding Properties Studies

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The release control of the transcription factor NF- κ B through the inhibition of the IKK complex represents a potential target for the modulation of immune function in the treatment of autoimmune diseases.^[1] The IKK complex is composed of two catalytic subunits (IKK1 and IKK2) and one regulatory subunit named IKK- γ or NEMO. Because of the central role played by IKK2 in response to apoptotic or inflammatory stimuli, its inhibition is considered a promising approach for the treatment of chronic inflammation and cancer. However, inhibition of IKK1 may promote severe side effects.^[2] Therefore, the identification of differing structural features between these two kinases is very important for the development of novel selective IKK2 inhibitors, candidates for anti-inflammatory and anticancer drugs. In this work, we used comparative modeling to obtain a three-dimensional structure of IKK2 (and also IKK1) and performed molecular docking studies to achieve a better understanding of the molecular properties that may confer potency and selectivity to some inhibitors.

The molecular models were constructed (using Modeller v.8 software) through a multiple templates approach. The molecular docking studies of the cofactor ATP (used as control reference to access the model quality for docking studies) and 20 published inhibitors with various potencies and selectivities against IKK1 and IKK2 were performed with Glide v.5.7 software.

IKK1 and IKK2 have 61% sequence identity and share a common folding with other serine/threonine kinases. We observed that the main differences between IKK1 and IKK2 is near to the hinge region (Ser85 in IKK to Gln86 in IKK2) and in the N-lobe (Leu17 in IKK1 to Arg17 in IKK2), but do not seem to be important for molecular recognition of the ATP cofactor. Molecular docking results indicated that 1) the potency of the stronger inhibitors can be associated with

the formation of one or more hydrogen bonds between the ligand and the hinge region, mainly with Cys85; and 2) the selectivity of the inhibitors could be associated with the presence of aromatic groups at the final part of the hinge region. Previous work showed that the compound LASSBio-15243 was selective for IKK2 (IC₅₀ IKK1: 0% and IC₅₀ IKK2: 20000 nM), but docking studies were unable to reveal a binding mode consistent with experimental results. In this work, we were able to predict the binding mode of this compound relative to the IKK2 active site, and we concluded that: 1) the nitro group points toward the hinge region, but does not interact directly (i.e. does not make hydrogen bonds) with important residues in this region; 2) the molecule is stabilized by a π -stacking interaction with Phe12 and Trp44; and 3) the *N*-acylhydrazone group makes hydrogen bonds with Lys30 and Asp152, which are highly conserved in the kinase family.

The results obtained indicate that the use of the constructed model of IKK2 in the molecular docking experiments, validated through ATP docking, was able to provide important information regarding the potency and selectivity of the inhibitors tested. The development of a new compound (LASSBio-1760, already synthesized) was guided by analyses of the docking results of IKK2 potent inhibitors and LASSBio-1524.

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Modification of Thiazolinone Derivatives as Potent 5-Lipoxygenase Inhibitors

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Leukotrienes (LTs) belong to a topical class of bioactive lipid mediators that are generated by oxygenation and further conversion of arachidonic acid. One key enzyme of their biosynthesis is an iron-containing, heme-free dioxygenase: 5-lipoxygenase (5-LO). LTs play a pivotal role in inflammation, allergic disorders, asthma, cardiovascular diseases, and cancer. Only two LT-interfering drugs are currently marketed: the LT receptor antagonist montelukast (Singulair®) and the iron-chelating 5-LO inhibitor zileuton (Zyflo®). Therefore, the need to find new active agents for anti-leukotriene therapy is still urgent.^[1,2]

Based on early virtual screening^[3] and medium-throughput screening we previously identified 5-arylidene-2-arylthiazolinone as a lead structure (Figure 1).^[4,5] Our previous structure–activity relationship (SAR) studies indicated that this lead may show a continuous SAR for 5-LO.^[4] We were next focused on the further evaluation, characterization and cytotoxicity of the thiazolinones. Therefore, we designed

and synthesized novel 5-arylidene-2-arylthiazolinone derivatives as direct 5-LO inhibitors, modifying electronic properties, lipophilicity, and introducing bicyclic or aryl residues (parts A–C). The compounds were prepared by one-pot domino reaction of thioglycolic acid and the corresponding benzaldehyde and benzonitrile in a related two-step synthetic procedure.

With the results of the 5-LO inhibitory assay using cell-free and whole-cell (PMNL cells)^[4,5] conditions, we identified a naphthalene-containing compound with a tenfold higher inhibitory potency than zileuton, and gained further insight into the flat SAR of the thiazolinone-based 5-LO inhibitors.

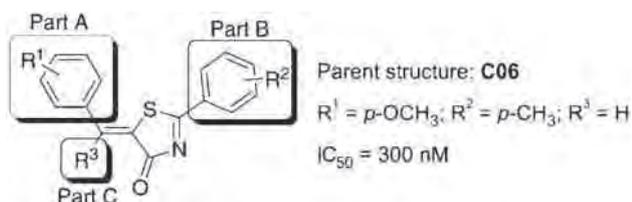


Figure 1. 5-Arylidene-2-arylthiazolinone core scaffold with structural variations.

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P291

Peptide Paratope Mimetics of Anti-HIV-1 Antibodies: A New Class of Promising Antiviral Agents

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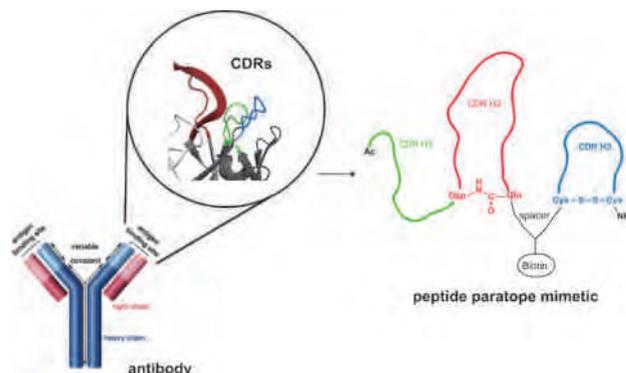
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During an infection of host cells with the human immunodeficiency virus (HIV), the virus first contacts the cell via its glycoprotein gp120, which binds to the CD4 receptor on the human cell surface.^[1,2] This causes conformational changes within gp120,^[3] exposing a conserved binding site, which enables binding of gp120 to one of the human co-receptors,^[4] CCR5 or CXCR4,^[5] and results, along with downstream events, in the invasion of the host cell.

To date, various broadly and strongly neutralizing antibodies that interfere with this infection process have been isolated from infected individuals.^[6] Based on the antibody structures in complex with their viral antigens (CD4 and co-receptors, respectively), we have designed and generated, by solid-phase synthesis, peptides that mimic the

paratopes, i.e. the antigen binding sites of some of these antibodies. These paratope mimetics, which are complex assembled peptides presenting the relevant CDR loops of the antibodies, were found to specifically bind to their target antigens in ELISA, as well as inhibit HIV infection in cellular infection assays, demonstrating the virus neutralization potential of antibody paratope mimetics.



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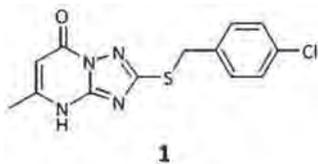
Factors Influencing the Specificity of Inhibitor Binding to the Human and *Plasmodium* Dihydroorotate Dehydrogenases

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A crucial aspect of drug discovery is the development of enzyme inhibitors that can effectively select between enzyme homologues: a requirement for both efficacy and safety. Dihydroorotate dehydrogenase (DHODH) is a metabolic enzyme that plays a crucial role in the biosynthesis of nucleic acids. Human dihydroorotate dehydrogenase (HsDHODH) is an established therapeutic target used in the treatment of rheumatoid arthritis.^[1] In addition, *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) is of current interest for the development of antimalarial drug candidates.^[2] We wished to elucidate the structural requirements for compounds to differentiate between the human and plasmodial enzymes.

Using a shape-similarity-based approach^[3] applied to an existing inhibitor scaffold,^[2] a novel inhibitor of both *Hs*DHODH and *Pf*DHODH was identified and found to be 50-fold more selective for *Pf*DHODH (compound **1**).



A SAR study was undertaken to examine what effects varying the substitution pattern of the benzyl group of compound **1** has on species selectivity. A library of 18 analogues including both mono- and di-substituted benzyl groups were synthesized and showed that minor alterations in substitution pattern could have a dramatic effect on the binding affinity of these compounds (see Table 1 for selected examples).

Compd	Ar	IC ₅₀ ± SE [μM]	
		<i>Pf</i> DHODH	<i>Hs</i> DHODH
1	4-chlorophenyl	1.0 ± 0.1	39 ± 2
2	2-chlorophenyl	28 ± 4	12 ± 2
3	3-chlorophenyl	5.6 ± 0.3	29 ± 1
4	4-bromophenyl	33 ± 6	32 ± 6
5	2,6-dichlorophenyl	3.5 ± 1.4	18 ± 3
6	2,5-dichlorophenyl	11 ± 1	0.051 ± 0.007
7	3,5-dimethylphenyl	19 ± 4	30 ± 5

The most pronounced effects are observed in the disubstituted compounds **5–7**, where selectivity can be switched from one species to another by the movement of one chlorine substituent (compounds **5** and **6**). To better understand this sensitivity, *in silico* modelling^[4] and crystallography were used to examine the binding poses of these compounds, leading to the discovery of a novel conformation of human DHODH. This work has highlighted how subtle changes in structure can have a profound effect on the selectivity and efficacy of ligands. Such knowledge will ultimately lead to the development of safe and more efficacious medicines.

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On the Trail of New Corticotropin-Releasing Factor-1 Antagonists: New Pyridazine Derivatives as Anxiolytic Agents

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Over 20 years of preclinical studies point to corticotropin-releasing factor (CRF) playing a role in the mediation of endocrine and behavioral responses to stress, as a consequence of the critical coordination carried out by CRF in the hypothalamic–pituitary–adrenal (HPA) axis.^[1] Clinical studies have supported a model of CRF dysfunction in depression and more recently a potential contribution to specific anxiety disorders. CRF-1 receptor antagonists constitute an emerging class of therapeutic agents that promise conceptually new strategies toward treatments, not only in the field of neuropsychiatry but also for other disorders such as irritable bowel syndrome,^[2] and craving and anxiety symptoms in cases of alcohol addiction.^[3] Nevertheless, a series of derivatives of the two well-known topologies of CRF antagonists, with pexacerfont and GW876008 as the most promising candidates, completed phase I/II clinical trials, not demonstrating efficacy.^[4]

During screening of our corporate molecular library, amino-functionalized pyrido[4,5-*d*]pyridazine was identified as a novel scaffold with CRF-1 antagonist activity. On this basis, a synthetic methodology leading to a number of derivatives was developed. Tested as CRF-1 inhibitors, these derivatives exhibited affinity in the nanomolar range (K_i : 5–56 nM; Table 1).

#	K_i [nM]
F90515OT	56
F00612OT	29
F00613OT	22
F00616OT	32
F90805OT	13
F00606OT	56
F80909TH	5

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Target-Guided Synthesis of Irreversible Protein Kinase Inhibitors

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The development of small organic molecules targeting protein kinases holds great promise to regulate unwanted kinase activities, both in medicinal chemistry and chemical biology research. The first steps of a target-guided approach for the development of new irreversible kinase inhibitors for a biologically uncharacterized mitogen-activated protein kinase are reported. The synthesis takes advantage of the biological target molecule that is used as a template for the construction of its own inhibitor through self-assembly of inhibitor-fragments within the target's active site. In this approach, a cysteine residue in the active site of the target kinase is covalently modified with a tether molecule that has an inherent affinity for the kinase. A library of reactive compounds is then added, and a proximity-enhanced reaction between the tether molecule and the library compounds that make favorable contacts with the active site of the kinases near the tether can take place. The hit compounds formed in this reaction can then be identified by mass spectrometry.

The target-guided synthesis approach combines the benefits of covalent tethering with combinatorial chemistry. This is a promising approach for the development of new kinase inhibitors, as inhibitor assembly and screening of compound libraries are accomplished in a single step, which makes the analysis of a large chemical space feasible. Furthermore, the development of irreversible kinase inhibitors is thought to address the emerging problem of acquired drug resistance mutations in targeted cancer therapies as well as limited selectivity of conventional kinase inhibitors.^[1,2]

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A Direct Binding Assay for the Detection of Allosteric Inhibitors of Abl

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The Abelson (Abl) tyrosine kinase is an important cellular enzyme that is rendered constitutively active in the breakpoint cluster region (BCR)–Abl fusion protein, contributing to several forms of leukemia.^[1,2] Although inhibiting Abl activity with the small organic molecule imatinib showed great clinical success, many patients acquire secondary mutations in BCR–Abl, resulting in resistance to imatinib.^[3] Second-generation kinase inhibitors such as dasatinib and nilotinib also fail to treat patients with the especially prevalent mutation T315I at the gatekeeper position of the kinase domain.^[4] A combination of the allosteric (type IV) kinase inhibitor GNF-2 with type II inhibitors was recently shown to overcome this mutation in Abl.^[5] This recent success highlights the need for identifying novel type IV inhibitors to perturb the kinase activity of the clinically relevant mutant variant Abl-T315I.

In this study, we developed a direct binding assay for the detection of type IV inhibitors of Abl by covalently attaching an environment-sensitive fluorophore close to the myristate binding site of the kinase domain. We show that this assay is a strong tool for the exclusive detection of ligands that bind to the myristate pocket, inducing the conformational changes characteristic for inactive Abl, whereas other ligands such as classic type I and II inhibitors are not detected. We then optimized the assay for high-throughput screening and for compounds with strong intrinsic fluorescence.

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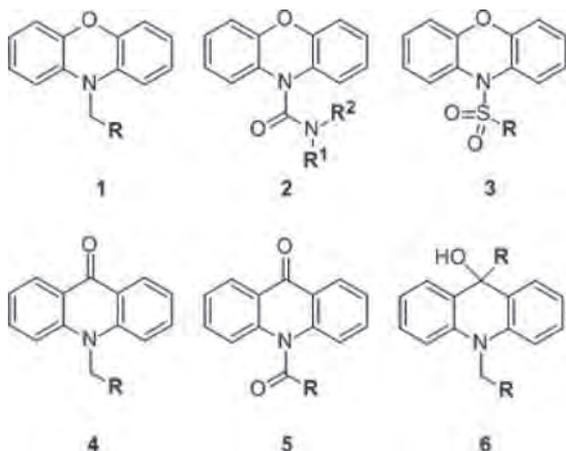
N-Substituted Phenoxazine and Acridone Derivatives: Structure–Activity Relationships of Potent P2X4 Receptor Antagonists

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ATP is an important signaling molecule, activating G-protein-coupled (P2Y) receptors and ligand-gated ion channels (P2X receptors). P2X receptors are trimeric ion channels, and seven subunits, P2X1–7, are known to exist, which form homo- or heteromeric ion pores permeable to Ca²⁺ and Na⁺ ions.^[1] The P2X4 receptor is expressed in various CNS areas, on immune cells, and on peripheral macrophages.^[1] The location and up-regulation of the P2X4 receptor selectively in spinal and/or supraspinal, injury-induced, activated microglia link this receptor to pathophysiological processes such as persistent and neuropathic pain, traumatic brain injury, cerebral ischemia, and spinal cord injury.^[2] Only very few P2X4 receptor antagonists are known to date, and no systematic structure–activity relationship (SAR) studies have been published yet.



We identified phenoxazine derivatives as a novel class of P2X4 receptor antagonists and investigated the SARs of N-substituted derivatives as well as those of related acridones (see general structures 1–6). The compounds were evaluated in Ca²⁺-flux assays at 1321N1 astrocytoma cells recombinantly expressing the human P2X4 receptor. Selected compounds were further investigated at rat P2X4 receptors for potential species differences, and at the other homomeric P2X receptor subtypes. In addition, radioligand binding assays using [³⁵S]ATPγS were performed for selected ligands to investigate whether they interact with the ATP binding site of the P2X4 receptor. One of the most potent P2X4 receptor antagonists of the present series was 10-[(4-methylphenyl)sulfonyl]-10H-phenoxazine (scaffold 3), showing IC₅₀ values of 1.38 μM (human), 0.928 μM (rat), and 1.76 μM (mouse) P2X4 receptors; the compound showed high selectivity

versus other P2X receptor subtypes (P2X1, P2X2, P2X3, and P2X7). Thus, it may be a useful pharmacological tool, and represents a lead structure for the development of novel drugs.

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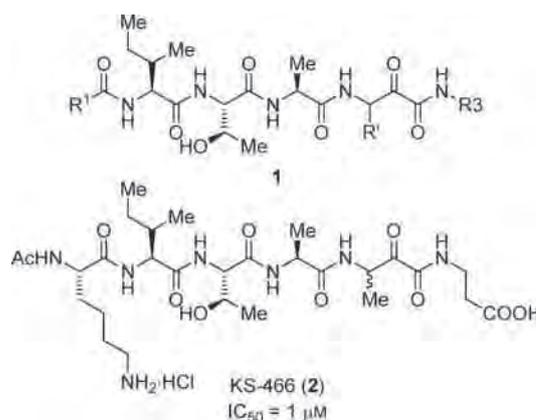
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Peptidic α-Ketoamide-Based PfsUB1 Inhibitors

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PfsUB1 is a malarial subtilisin-like serine protease that is required for the exit of merozoites from infected erythrocytes.^[1,2] Due to this function, PfsUB1 appears to play a critical role in the life cycle of the parasite, making PfsUB1 an attractive target for the development of novel antimalarial drugs.^[2] Despite considerable efforts to discover PfsUB1 inhibitors, no active small-molecule inhibitor has been reported until now. We designed peptidic ketoamides **1** as potential inhibitors^[3] of PfsUB1 based on the best known substrate sequence KITAQ/DDEES.



Synthetic routes toward compounds of general structure **1** were developed, and a small set of analogues was prepared, displaying low-micromolar inhibitory activity in the PfsUB1 assay. Analysis of substrate specificity studies and homology modeling of PfsUB1 indicated that acidic residues are preferred for the prime side of the enzyme. This promoted further optimization of the structure, leading to compound **2** (KS-466) with increased inhibitory activity (IC₅₀: 1 μM).

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P298

Synthesis and Pharmacological Evaluation of Huprine-Based Multitarget Anti-Alzheimer Compounds

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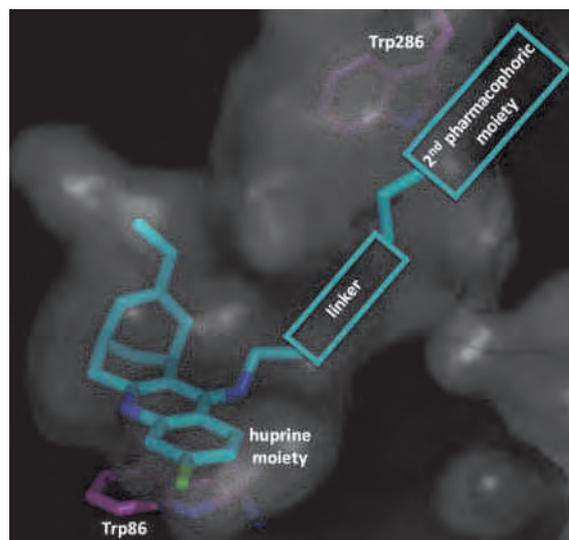
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To date, an effective curative or preventive therapy for Alzheimer's disease (AD) still remains elusive. The difficulty in developing an effective therapy for AD lies in the fact that it results from multiple molecular defects, i.e. the formation of senile plaques and neurofibrillary tangles, neurotransmitter deficiencies at the CNS, extensive oxidative stress, and inflammation. The multifactorial nature of AD might make drugs that hit a single target inadequate for its treatment. For this reason, the use of compounds that simultaneously hit multiple molecular targets involved in disease pathogenesis should be associated with increased efficacy and safety relative to single-target therapeutic interventions.

Herein we report the synthesis and pharmacological evaluation of a series of huprine-based hybrids which, *in vitro*, have proven to hit several key targets involved in the neuropathogenesis of AD, namely acetylcholinesterase (with dual-site binding), butyrylcholinesterase, β -secretase (BACE-1), and β -amyloid aggregation.



Acknowledgements: Support from DGICYT (CTQ2008-03768/PPQ) and Generalitat de Catalunya (2005SGR00180, 2009SGR1396) is gratefully acknowledged.

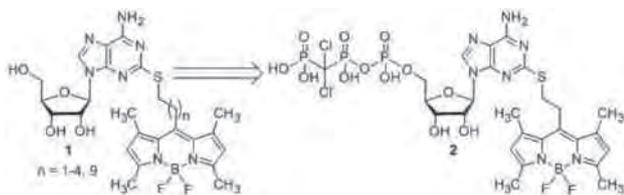
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Synthesis of New Fluorescent BODIPY-Labeled Nucleosides and Nucleotides as Molecular Sensors for Studying Purine Receptors

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The development of fluorescent receptor ligands as accurate and safe tools for studying receptor binding and function is of considerable interest.^[1,2] In the present study we developed fluorescent purinoreceptor ligands containing 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-S-indacene (BODIPY) as a fluorescent moiety. BODIPY fluorophores are useful tools to image processes in living cells due to their high photochemical stability, exceptional spectral properties, high absorption coefficients, and small size. The newly synthesized BODIPY derivatives fluoresce at ~500 nm and therefore do not interfere with biological fluorophores; they are therefore highly suitable for biological investigations.^[2,3] To obtain tools for studying adenosine receptors, BODIPY was attached to 2-thioadenosine via alkyl spacers of various lengths, as shown below (general structure **1**). The affinities of the obtained derivatives were determined in radioligand binding studies at adenosine A₁, A_{2A}, A_{2B}, and A₃ receptors. Some of the BODIPY-coupled nucleosides showed nanomolar affinities for A₁, A_{2A}, and A₃ receptors. The derivative with a short linker (*n*=1) showed receptor-subtype selectivity for A₃, whereas a longer alkyl chain (*n*=9) led to a preference for A₁ receptors.



As a fluorescent nucleotide tool for probing adenylate cyclases, we previously synthesized a metabolically stable, fluorescent 2'-MANT-ATP analogue, stabilized by a Pb–Pg–dichloromethylene bridge.^[4] We also obtained the ATP analogue [³H]2-propylthio–Pb–Pg–dichloromethylene-ATP ([³H]PSB-0413) as a high-affinity P2Y₁₂ receptor radioligand.^[5] In the present study, we attached the fluorescent BODIPY moiety to the 2-position of the metabolically stable Pb–Pg–dichloromethylene-ATP via an ethylthio linker, yielding nucleotide **2**. The affinity of **2** at ATP receptors P2X_{1–4} was investigated in radioligand binding studies versus [³⁵S]ATPyS. The potency of **2** was also determined in cAMP assays at P2Y₁₁ receptors. Finally, the affinity of **2** was investigated in radioligand binding assays at P2Y₁₂ receptors using [³H]PSB-0413. The fluorescent nucleotide **2** showed high affinity (0.33 μM) for this receptor. The new fluorescent purinoreceptor ligands will be useful tools for studying purine receptor binding and function.

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P301

Design, Synthesis and Evaluation of α₂-Adrenoceptor Antagonists: Towards New and Improved Antidepressants

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The α₂-adrenoceptor (α₂-AR) has been identified as a promising target for the treatment of depression. It has been found in increased density and hyperactive conformation in the brain of depressed suicide victims during autopsy.^[1] Activation of the α₂-AR decreases levels of noradrenaline in the brain, an effect associated with depression. Furthermore, its activation has been implicated in the aetiology of decreased hippocampal neurogenesis and volume which now are seen as fundamental causative factors of depression.^[2]

Therefore, antagonists of the α₂-AR are attractive targets to treat depression. Unfortunately, the crystal structure of the α₂-AR—a GPCR—has not been elucidated, precluding a structure-based design of antagonists of the receptor. Existing ligands vary widely in structure and activity at the receptor, and very structurally similar molecules from our research group have given both agonistic and antagonistic activity at the receptor.

This project focuses on an analogue-based drug design strategy to clarify the requirements for binding to and antagonism at the α₂-AR. An ab initio density functional theory study of proposed ligands, coupled with NMR and crystal structure data, has allowed important structure–activity relationships to be drawn from biological testing. Significantly, our molecules displayed only the desired antagonistic or inverse agonistic activity at the α₂-AR during [³⁵S]GTP-γS functional binding assays in human prefrontal cortex tissue (Figure 1). Furthermore, we have identified and computationally verified a number of molecular properties which lead to increased binding affinity at the α₂-AR in competitive binding experiments with the radiolabelled α₂-AR ligand [³H]RX821002.

Herein we present the results from this work,^[3] along with progress in the synthesis and testing of subsequent families of molecules which have stemmed from the positive biological results of families 1 and 2, as well as other ongoing research within the Rozas group.

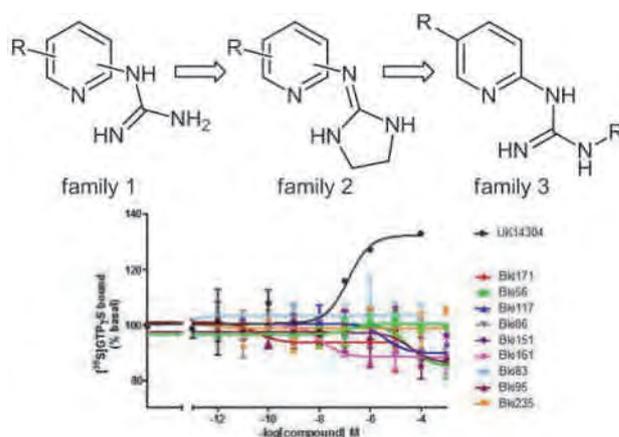


Figure 1. Families 1, 2, and 3 (top) and antagonistic/inverse agonistic activity of family 1 compared with standard agonist UK14304 (bottom).

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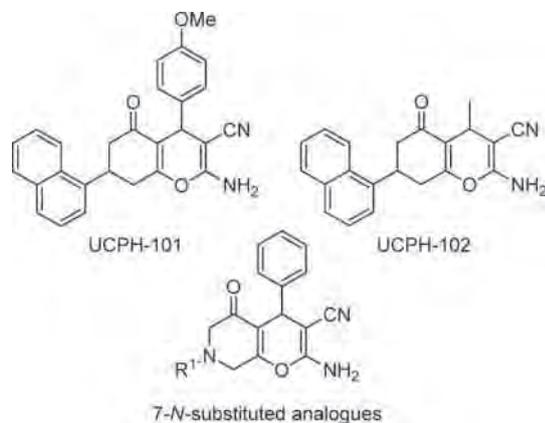
P302

Structure–Activity Relationship Study of Selective EAAT1 Inhibitor UCPH-101 and Absolute Configurational Assignment Using Infrared and Vibrational Circular Dichroism Spectroscopy in Combination with Ab Initio Hartree–Fock Calculations

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Excitatory amino acid transporters (EAATs) play a pivotal role in regulating the synaptic concentration of glutamate in the central nervous system (CNS). To date, five different subtypes, named EAAT1–5, have been identified in humans. In rodents their naming is different for historic reasons: GLAST, GLT-1, EAAC1, EAAT4, and EAAT5, respectively.^[1] We recently published and presented a structure–activity relationship (SAR) study of the first class of selective inhibitors of EAAT1 (and GLAST), with the analogues UCPH-101 (IC₅₀=0.66 μM) and UCPH-102 (IC₅₀=0.43 μM) being the most potent inhibitors in the series.^[2,3] Comprising two chiral centers, UCPH-101/102 were synthesized and characterized pharmacologically as a mixture of four stereoisomers; however, the inhibitory activity resides in only two of these. Herein we present the design, synthesis, and pharmacological evaluation of seven 7-N-substituted analogues of UCPH-101/102.^[4] Of these, the absolute configurations of enantiopure 7-N-substituted analogues were determined by VCD in combination with ab initio Hartree–Fock calculations. The pharmacophore of this class of selective EAAT1 inhibitors was clarified further which will advance the future design and synthesis of selective EAAT1 inhibitors.



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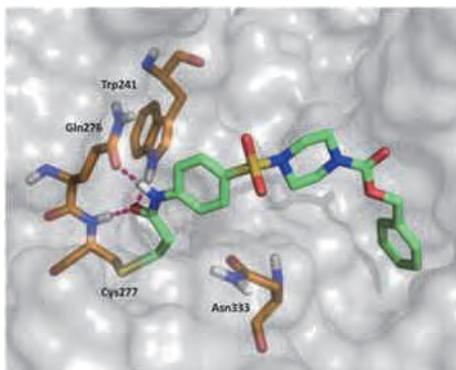
P303

Lead Optimisation of Potent and Selective Transglutaminase-2 Inhibitors for Huntington's Disease

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Tissue transglutaminase 2 (TG2) is a multifunctional protein primarily known for its calcium-dependent enzymatic activity of cross-linking proteins by isopeptide bond formation between glutamine and lysine residues.^[1] TG2 overexpression and activity has been found to be associated with Huntington's disease (HD) by several investigators.^[2,3] In addition, TG2 is known to deamidate and cross-link gluten-derived gliadin peptides, favouring the progression of celiac disease (CD).^[4] Herein we report a novel class of TG2 inhibitors that were developed from a nonselective fragment-like hit to analogues that display nanomolar potencies with desired selectivity profiles over the other TGase isoforms. We also report detailed in vitro DMPK profiling, subsequent development of a second-generation inhibitor with improved plasma stability, and assess its potential for further progression into proof-of-concept in vivo studies.



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P304

Computer-Aided Discovery of Novel Chemotypes Addressing the Chemokine Receptor CXCR3

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Traditionally, drug discovery efforts for GPCRs have been largely ligand-based. Recent determination of the X-ray structure of the chemokine receptor CXCR4 offered us an opportunity to initiate a homology-model-based approach to identify novel chemotypes addressing the related chemokine receptor CXCR3. The chemokine receptor CXCR3 is a G-protein-coupled receptor involved in regulating the functions of the immune system in health and disease. Any malfunctioning of CXCR3 signaling can lead to autoimmune diseases (e.g., multiple sclerosis and rheumatoid arthritis), cancer, and transplant rejection. Consequently, CXCR3 is a very attractive pharmacological target.

We used the recently resolved X-ray structure of CXCR4 to build a homology model of CXCR3. The obtained homology model of CXCR3 was refined by using a library of 600 known CXCR3 ligands with different affinities and non-binders. After several iterations of model refinement, the homology model demonstrating the best docking score for the binding of AMG487 was used for virtual screening. We used ZINC, a free database of commercially available compounds for virtual screening. Nearly one million “lead-like” molecules were docked, and candidate compounds were selected from the 500 best-ranked molecules after visual inspection. These molecules were tested in a functional assay measuring CXCR3-mediated [³⁵S]GTPγS incorporation and in a radioligand displacement assay using a novel

radiolabeled allosteric modulator of CXCR3 named RAMX3. This radioligand was developed based on an 8-azaquinazolinone core to facilitate the discovery of novel CXCR3 ligands that share the binding pocket with RAMX3 and its derivatives like AMG487. Six novel chemical scaffolds modulating the function of CXCR3 were discovered. Interestingly, although we refined the homology model with negative allosteric modulators of CXCR3, we also identified positive (PAM) and silent (SAM) besides negative (NAM) allosteric modulators, indicating the delicate balance between a molecule’s function as SAM, NAM, or PAM. The best identified PAM that increased CXCL11-mediated activation of CXCR3 had a K_b value of 127 nM and an α value of 1.95. The best NAM that decreased CXCL11-mediated activation of CXCR3 had a K_b value of 196 nM and an α value of 1.10. One compound acting as SAM exerted no activity in the functional assay, but was able to suppress the binding of radiolabeled allosteric modulator RAMX3 with a K_i value of 15 μ M. SAMs are useful molecular scaffolds for the development of novel modulators. Even slight chemical modifications of SAMs can switch them to functionally active NAMs or PAMs.

Our investigations demonstrate that a carefully refined homology model can provide a productive template for the discovery of novel chemotypes for allosteric modulators even for such challenging targets as chemokine receptors.

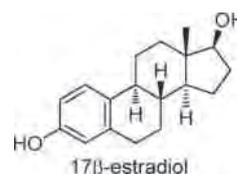
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Synthetic Strategies to Afford Estradiol Derivatives with Potential Biological Activity

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Estrogens are a group of steroid hormones that exhibit diverse mechanisms of action in multiple physiologic systems and are also implicated in the development or progression of numerous diseases, such as various types of cancer (breast, ovarian, prostate), osteoporosis, neurodegenerative and cardiovascular diseases.^[1] Traditionally, the actions of estrogens, in particular 17 β -estradiol (E2, shown below), are associated with two nuclear estrogen receptors (ERs), ER α and ER β , which function as ligand-activated transcription factors. However, E2 also mediates rapid signaling events via pathways that involve transmembrane ERs, such as G-protein-coupled ER 1 (GPER; GPR30). In the past 10 years, GPER has been implicated in both rapid signaling and transcriptional regulation, and the discovery of GPER-selective ligands became a new field in medicinal chemistry, both with new compounds and with new studies in the “old players”.^[2]



Within our framework of new reactions and processes toward bioactive steroids,^[3,4] we have been exploring the selective modification of key positions of the estradiol scaffold to prepare new derivatives. Herein we report synthetic modifications at the A and D rings of the steroid skeleton. Modifications on hydroxy groups at positions 3 and 17 were performed with good yields. Diols and β -hydroxy ether derivatives were obtained from selective ring opening of epoxides. The use of lipases in organic media to prepare the corresponding monoacylated derivatives starting from either *cis*- or *trans*-diols are discussed. The relative binding affinity and intrinsic activity of each test compound toward the nuclear and membrane-associated ERs will be evaluated in vitro using pharmacological approaches and selective functional assays in cell lines differentially expressing those receptors.

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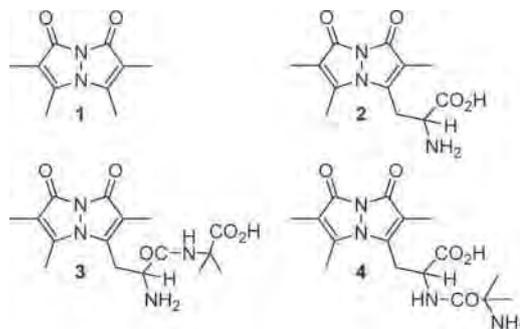
P306

The Synthesis of Fluorescent Amino Acids Based on a *syn*-Bimane Moiety and Their Incorporation in Dipeptide Mimics

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The ability to introduce fluorophores^[1,2] selectively into proteins provides a powerful tool to study protein structure, dynamics, localization, and biomolecular interactions both in vitro and in vivo. Herein we report a strategy for the selective and efficient synthesis of a novel low-molecular-weight fluorophore based on *syn*-bimane^[2,3] unit **1**, which is effective at different wavelengths^[4] (absorption maximum at $\lambda=480$ nm [$\epsilon=2500$] and nearby emission at $\lambda=450$ –600 nm), as compared with the naturally occurring aromatic amino acids like tryptophan, tyrosine, and phenylalanine present in naturally occurring polypeptides. Those show an absorption maximum at $\lambda=280$ nm and emission at $\lambda=300$ –350 nm.^[5]



We also report on the incorporation of **1** into dipeptide mimics. The reaction of *syn*-monobromobimane with diethyl acetamidomalonate under basic conditions afforded a fluorescent acetamido diethyl ester derivative. Hydrolysis and decarboxylation under acidic conditions gave *syn*-bimane-amino acid conjugate **2**. Coupling of **2** with methylalanine afforded the fluorescent dipeptide mimics **3** and **4**, which can be applied in brain research.

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P307

H/D Exchange in Dicationic Imidazolium Systems

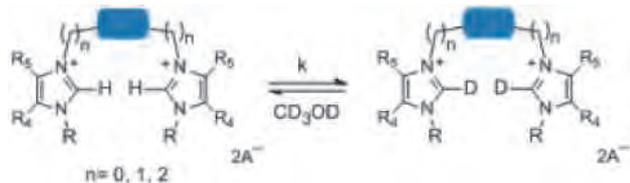
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H/D exchange reactions at carbon centers are of interest in many respects, whether it be for the preparation of isotopically labeled compounds, in basic research on C–H bond activation, or in mechanistic investigations on catalysts and reaction pathways. Isotope exchange experiments are capable of examining structural and reactivity features of synthetic as well as biological hydrogen bonded supramolecules, as hydrogen atoms involved in bonding interactions usually behave differently from freely accessible ones.

Imidazolium-based systems are becoming increasingly useful in a widening range of fields that include anion recognition chemistry, ionic liquids, and *N*-heterocyclic carbenes (NHCs).^[1,2] Moreover, of great interest to chemists are the kinetic acidity of imidazolium cations that include H/D exchange rates, carbon–proton acidity, and carbene precursor stability,^[1] as well as biologically active NHC complexes and their medicinal application.^[3,4]

Herein we report the H/D exchange rates of the C(2)–H of several bis(imidazolium) dications in $[D_4]$ methanol. In addition, the influence of the counterion, concentration, and presence of D_2O was studied. The observed exchange rates might give a rationale for the suitability of imidazolium salts as hydrogen bond donors or precursors of NHCs.



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Bioactivation of Bazedoxifene and 2-(4-Hydroxyphenyl)-3-methyl-1H-indol-5-ol in Human Liver Microsomes

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Bazedoxifene is a selective estrogen receptor modulator (SERM) that has been developed for use in post-menopausal osteoporosis. However, it contains a potentially toxic 5-hydroxy-3-methylindole moiety. Previous studies on the 5-hydroxyindole- and 3-alkylindole-containing drugs indometacine, zafirlukast, and MK-0524 structural analogues have shown that they are bioactivated by cytochrome P450s through a dehydrogenation process to form quinoneimine or 3-methyleneindolenine electrophilic species.

We showed that bazedoxifene is bioactivated only in trace amounts with recombinant CYP isozymes. In contrast, the N-dealkylated fragment of bazedoxifene (2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol) was bioactivated in considerable amounts to an electrophilic intermediate, which was trapped with glutathione and identified by LC–MS/MS. This suggests that bazedoxifene would require initial N-dealkylation, which could subsequently lead to the formation of the reactive intermediate. However, such an N-dealkylated metabolite of bazedoxifene was not detected after incubation of bazedoxifene in HLM or recombinant CYP isozymes.

We have confirmed that bazedoxifene, a new, third-generation, indole-based ER ligand, which has been developed for use in post-menopausal osteoporosis, offers an improved safety profile over currently available SERM therapies. Bazedoxifene was not dehydrogenated with recombinant CYP450 isozymes or HLM to form reactive electrophilic species, and is therefore unlikely to cause adverse effects by covalently binding to the nucleophilic residues of proteins and/or DNA. The results of the studies with bazedoxifene and its structural 5-hydroxy-3-methylindole-based fragment, coupled with several reports on other 3- and 5-substituted indole-containing drugs, provide additional evidence that this aromatic moiety should be used with caution in the development of new therapeutic agents. This study provides further proof that not all compounds possessing a potential structural fragment for bioactivation (structural alert) will necessarily, under bioactivation, elicit the formation of reactive species in vitro.

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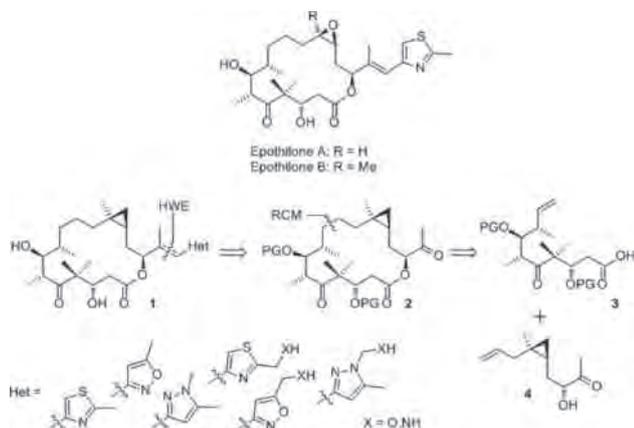
A New Synthetic Approach to Side-Chain-Modified Analogues of Cyclopropyl-Epothilone B

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Epothilones (Epos, see scheme) are microtubule-stabilizing agents with potent antitumor activity.^[1] Initially isolated from the myxobacterium *Sorangium cellulosum* with Epo A and B as the major variants, epothilones have served as important lead structures for anticancer drug discovery.^[1] Among numerous other modifications, replacement of the epoxide ring by a metabolically more stable cyclopropane moiety has been shown to be well tolerated, and the same is true for a variety of side chain modifications. In a project that aims at the construction of antibody–drug conjugates, we have now prepared a series of side-chain-modified analogues of cyclopropyl-Epo B **1**, and we evaluated their antiproliferative activity.

The synthesis of analogues of **1** is based on a novel, flexible approach toward the cyclopropyl-Epo B scaffold that relies on late-stage introduction of the side chain through HWE chemistry and ring closure by RCM (see scheme). This contribution discusses the synthesis of macrolactone **2** from building blocks **3** and **4** and its elaboration into the desired target structures. Preliminary results for a first antibody–drug conjugate will be presented.



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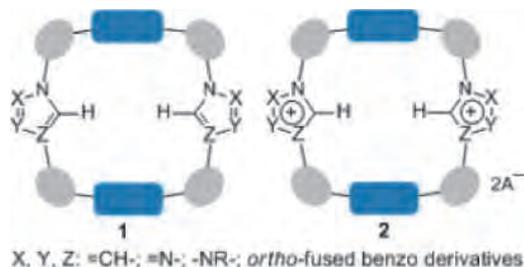
P310

Heterophane Prototypes as Sensors and Transporters

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The demand for anionic synthetic receptors has been increasing rapidly in the fields of transport and extraction of anions and sensing mechanisms due to the number of fundamental roles played by anions in biological and chemical processes. In the last few years, azolium and azole functionalities have gained a place among the anion binding functional groups and have emerged as attractive starting points for the design of abiotic anion receptors.^[1-4] This circumstance has given a biological perspective in the rapidly growing area of bionanotechnology, the aim of which is to develop new tools for biology, new biomaterials, selective sensors and supramolecular devices for clinical analysis, new therapeutics, and smart drug delivery systems. Continuing our research into azolium-based frameworks, herein we report the binding properties of heterophanes **1** and **2** with azole or azolium subunits as anion recognition motifs.



Acknowledgments: The authors thank the SCT-UB for use of their instruments, and AGAUR (*Generalitat de Catalunya*), *Grup de Recerca Consolidat 2009SGR562*.

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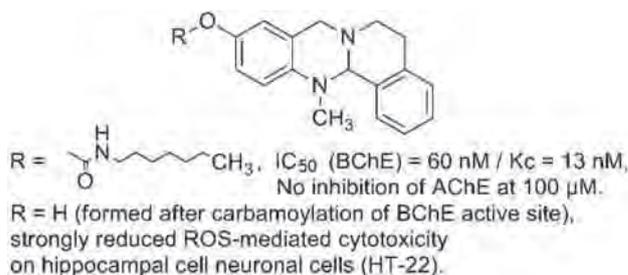
P311

Multi-target Tri- and Tetracyclic Pseudoirreversible Butyrylcholinesterase Inhibitors Releasing Reversible Inhibitors with Neuroprotective Properties upon Carbamate Transfer

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Tri- and tetracyclic nitrogen-bridgehead compounds were designed and synthesized to yield micromolar cholinesterase (ChE) inhibitors as starting points for structure–activity relationships (SARs) that identified potent compounds with butyrylcholinesterase (BChE) selectivity. In a subsequent step, these structures were used for the design and synthesis of carbamate-based (pseudo)irreversible inhibitors. Compounds with further improved inhibitory activity and selectivity were obtained and kinetically characterized, also with regard to the velocity of enzyme carbamylation. Structural elements were identified and introduced that showed additional neuroprotective properties on a hippocampal neuronal cell line (HT-22) after glutamate-induced generation of intracellular reactive oxygen species (ROS). We identified nanomolar and completely selective pseudoirreversible BChE inhibitors that release reversible inhibitors with neuroprotective properties after carbamate transfer to the active site of BChE.



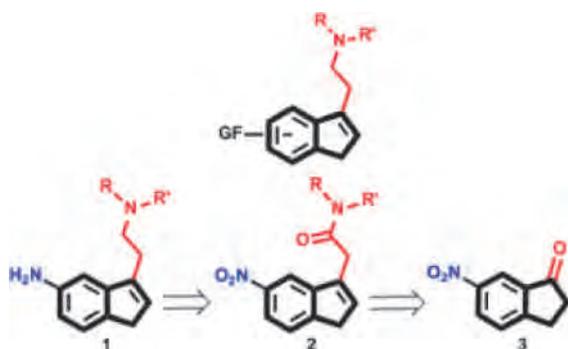
P312

Synthetic Approaches to Multifunctional Indenes

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Compounds with an indene core are of great interest as a source of bioactive compounds in drug discovery and development. In addition, indene-based structures are precursors of metallocene complexes for catalytic polymerization processes, as well as being present in *N*-heterocyclic carbene ligands and functional materials.



The synthesis of multifunctional indenes with at least two different functional groups has not yet been extensively explored. Among the plausible synthetic routes to 3,5-disubstituted indenes bearing two different functional groups such as the [3-(aminoethyl)inden-5-yl] amines **1**, a reasonable pathway involves the (5-nitro-3-indenyl)acetamides **2** as key intermediates. Although several multistep synthetic approaches could be applied to these advanced intermediates, we describe herein their preparation via an aldol-type reaction between 5-nitroindan-1-ones **3** and the lithium salt of *N,N*-disubstituted acetamides, followed immediately by dehydration with acid.^[1]

This classical condensation process, which is neither simple nor trivial despite its apparent directness, permits an efficient entry to a variety of indene-based molecular modules that could be adapted to a range of functionalized indanones.

Acknowledgements: Thanks are due to the AGAUR (Generalitat de Catalunya), Grup de Recerca Consolidat 2009SGR562.

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P313

DNA Conformational Properties: Browsing among G-Quadruplex States and Implications in the Drug Design of Selective Binders

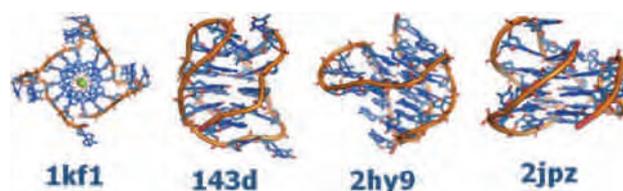
Stefano Alcaro, Anna Artese, Giosuè Costa, Federica Moraca, Francesco Ortuso, Lucia Parrotta

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DNA has long been considered a favored target for cancer chemotherapeutic agents.^[1] G-quadruplex folds have recently attracted the attention of drug designers due to their relevant role in pathological conditions,^[1] such as cancer and viral infections. The polymorphism of the G-quadruplex has been experimentally demonstrated in several environments^[3] and also evaluated in our research group by theoretical methods using the most active binder telomestatin as probe.^[4]

Because the G-quadruplex conformation can be obtained in different guanine-rich sequences, we considered the conformational characterization of this special DNA as an important goal by starting from the known telomeric structures deposited into the Protein Data Bank.

We analyzed them and started molecular modeling simulations with the aim to characterize the conformational profile of the telomeric target, including the interconversion from one to another fold. Several computational approaches have been adopted, allowing direct comparison between them and the identification of the most adequate protocol to apply with different DNA or RNA guanine-rich sequences. The results of this work will be useful for building new models for the rational drug design of novel selective G-quadruplex binders.



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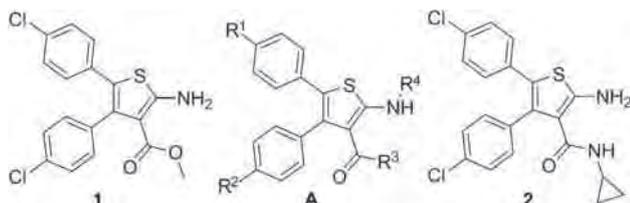
Synthesis, Biological Evaluation and Structure–Activity Relationship Studies in Disruptors of the p53–MDM2 Interaction Based on a 3,4,5-Trisubstituted Amino thiophene Scaffold

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The first identified tumor suppressor, p53, is a potent transcription factor which plays a key role in the induction of cell-cycle arrest and apoptosis. Proof-of-concept experiments have demonstrated that blocking the p53–MDM2 (a master negative regulator of p53) interaction can effectively reactivate wild-type p53 in tumor cells, leading to their death; this is now recognized as a promising therapeutic strategy for tumor treatment.^[1]

A 3,4,5-trisubstituted amino thiophene derivative **1** was identified by screening our in-house compound database based on a computationally derived pharmacophore model of MDM2 binding, which demonstrated both low-micromolar inhibition of the p53–MDM2 interaction and antiproliferative activities in tumor cell lines. This compound represents a novel lead compound for further p53–MDM2 inhibitor design. Careful SAR studies around generic structure **A** were developed by introducing a wide range of substituents at positions R¹–R⁴ positions to optimize the potency. Most of the 3,4,5-trisubstituted amino thiophene derivatives possessed potent MDM2 binding affinities and excellent antiproliferative activities in vitro. Additionally, the preliminary pharmacokinetic experiments showed that a representative compound (**2**) of these analogues had good stability in rat whole blood and human liver homogenate.



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P315

Pre-competitive PK/PD Profiling of GPCR Drug Candidates: High-Quality Data and Prediction Models with KNIME®

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Experimental PK/PD preclinical profiling plays a consistent and resource-intensive part of the drug discovery approach, both in industrial and in academic environments. Together with activity PoC in vivo, a satisfactory PK/PD profile has a dominant role in any due-diligence process in licensing or in any decision for a drug candidate toward early human clinical phase.^[1] Several toxicity prediction models have received attention from regulatory agencies, but few models have been published so far targeting precompetitive PK/PD profiling of candidate drugs.^[2]

In latest years, some non-commercial models/programs have been produced mostly with governmental funding and published.^[3] Most of them have dealt with metabolic oxidations, especially on CYP450s or hERG channel inhibition.^[4] However, there is a growing interest in web-based or open-source applications dealing with PK/PD data. Being active in high-quality data management services, herein we share our findings regarding predictive models on rat i.v. clearance (CL), half-life ($t_{1/2}$) and volume of distribution (V_{ss}), as we envisioned these models as integrated and determinant for a pre-competitive assessment of novel candidates^[5] where open-source software has led to a boost in recent years.

A sample of 235 GPCR-active compounds from our small-molecule ligand database^[6] was used. PK/PD published data (1997–2012) from rat i.v. subadministration experiments have been normalized by dose and modeled in classical regression approaches without success. We then classified CL, $t_{1/2}$, and V_{ss} data in binary classes (high/low) using, as reasonable thresholds for each parameter, 65 mL min⁻¹ kg⁻¹, 6 h, and 2.5 L kg⁻¹, respectively. After 70% sample partitioning in training/test sets through stratifications on the binary classes, four high-quality classification models for each parameter were produced with high Q^2 (30× leave-group-out) ranging from 0.71 to 0.88. Test sets were checked for their applicability to the model through nodes freely available in the KNIME 2.5.1 software package,^[7] which was used for workflow implementation of the study. The models with highest Q^2 values were produced for $t_{1/2}$ using SVM kernels (RBF or polynomial). Due to the large number of descriptors available, backward feature elimination was used. All twelve models are based on 3–12 descriptors, all of them coming from the set of another open-source chemoinformatic tool, RDKit.^[7] As relative most determinant descriptors chosen for the models point to atomic contributions to surface area,^[8] interpretations of these exciting results will also be discussed.

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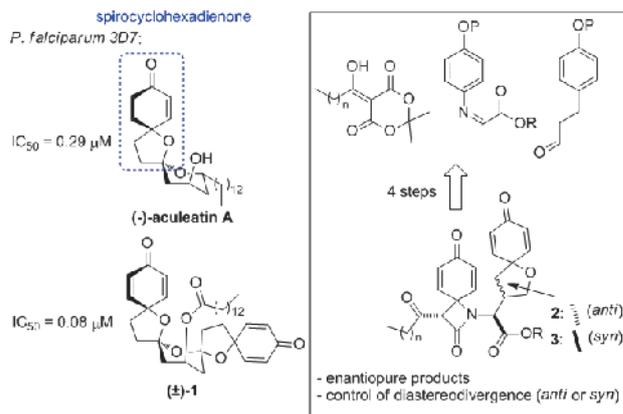
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Organocatalytic Strategy for the Rapid Construction of Stereogenic Diverse New Antimalaria Products

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Malaria is one of the most widespread parasitic infections in the world. The current spread of *Plasmodium falciparum* infection is mainly due to the emergence of resistance to virtually all available antimalarial drugs. Novel therapeutic chemotypes are therefore urgently needed. Inspired by a potent new class of antimalarial natural products isolated in 2000, the aculeatins, we were able to optimize a racemic analogue **1** a few years ago, possessing a double pharmacophoric scaffold (spirocyclohexadienone), acting at nanomolar concentrations and with a selectivity index >100 (efficiency on the parasite versus cytotoxicity on human erythroblasts, SI: 109–123).^[1,2] Concerned by the need to produce inexpensive and easily made drugs for patients who are located mainly in poor or developing countries, we have developed an organocatalytic approach to quickly and simply produce new sets of structurally complex enantiopure molecules with the essential features of antiparasitic agents (double pharmacophoric scaffold and a lipophilic chain) and displaying stereogenic diversities. Their biological evaluations on *P. falciparum* strains have given new insight into their structure–activity relationship.



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Design and Synthesis of Bivalent Ligands Targeting the NMDA/D1 Receptor Complex

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N-Methyl-D-aspartate (NMDA) receptors are critically involved in the majority of excitatory neurotransmission. They are implicated in numerous events in the brain and play an important role in the synaptic plasticity associated with memory and learning. The NMDA receptors are thought to be involved in several neuropathological conditions, including schizophrenia, and it is suggested that concurrent dysfunctions of glutamate and dopamine transmission may be the central event in the pathophysiology of this disease. Considerable evidence shows that NMDA receptors form receptor complexes with dopamine D1 receptors *in vivo*, and these NMDA/D1 receptor complexes may have physiological implications and therapeutic potential in the treatment of schizophrenia.

The overall aim of this project is to develop a set of chemical probes that can modulate the NMDA receptors in a specific manner. The compounds are designed to have increased affinity depending on whether the NMDA receptor is associated with the dopamine D1 receptor. This will be done by creating bivalent ligands targeting two different binding sites (Figure 1A).

Suitable ligands for linking strategies will be developed from known ligands for the NMDA and dopamine D1 receptors. An indole-carboxylate NMDA receptor glycine site antagonist and a clozapine derivative dopamine D1 receptor antagonist (Figure 1B) were chosen

as two of the ligands. Docking experiments and/or SAR studies suggest that a linker can be attached in the indicated positions without affecting the activity of the compounds dramatically. The functional groups at the attachment points make it possible to use an amide link and a hydrazone link as connecting functions. PEG linkers of variable length will be used to connect the ligands to look for the best interaction with the target receptor complex. These linkers are flexible, which is essential to allow correct positioning of each ligand.

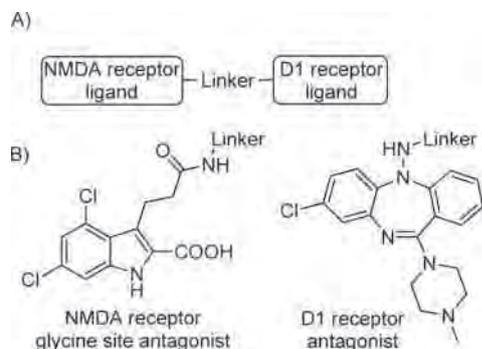


Figure 1. A) Schematic representation of the bivalent ligands. B) The structures of the NMDA receptor glycine site antagonist and the dopamine D1 receptor antagonist. The connection point to the linker is indicated in both structures.

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Design, Synthesis and Biological Evaluation of New Heterodimeric Derivatives Acting as Dual Binding Site Cholinesterase Inhibitors with β -Amyloid Antiaggregation Activity

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The multi-target-directed ligand design strategy is an attractive approach toward novel effective drugs for the treatment of disorders with complex pathological mechanisms such as Alzheimer's disease (AD). In recent years many multifunctional compounds like dual binding site cholinesterase inhibitors and/or inhibitors with additional properties such as β -amyloid anti-aggregating, antioxidant, neuroprotective, and voltage-dependent calcium channel antagonistic activity have been described.^[1] Therefore, there is reason to develop novel dual binding site cholinesterase inhibitors as multi-potent anti-AD agents.

The new series of heterodimeric compounds were designed according to fragment-based approaches. Some molecular fragments were docked into acetylcholinesterase (AChE) to find preferable interaction areas. They were then connected and optimized to obtain new derivatives with higher potency.^[2] All designed structures were also docked to butyrylcholinesterase (BuChE) to assess their activity against this enzyme. The novel compounds (Figure 1) were

synthesized and tested in Ellman's assay. Identification of β -amyloid antiaggregation activity for some cholinesterase inhibitors inspired interest in the evaluation of biological activity for our structures against this target in the thioflavin T test.

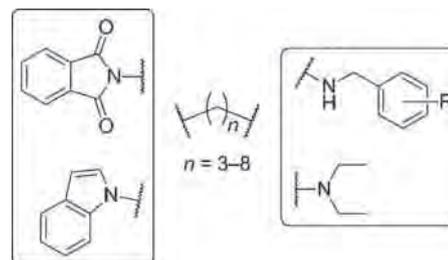


Figure 1. General structure of obtained dual binding site cholinesterase inhibitors.

Among the novel series, selective AChE inhibitors and inhibitors of both cholinesterases were disclosed. Their activities, expressed as IC_{50} values, ranged between 0.087 and 8.69 μ M for AChE and 1.06–11.22 μ M for BuChE. Some derivatives inhibited aggregation of β -amyloid by 22.72–41.27% at 50 μ M. The results obtained proved the molecular modeling method as a useful tool for the design of novel dual binding site cholinesterase inhibitors.

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P320

TGR5 Agonists Reduce the Production of Pro-inflammatory Th1 Cytokines

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Many inflammatory mediated diseases can be treated today, yet for many of them there is still an unmet need for alternative therapies that offer a benefit in terms of overall efficacy, ease of administration, and an improved side effect profile. To this end, significant research is being conducted toward identifying and dissecting selective biological pathways that are key drivers for human disease.

Although it has been known for some time that the G-protein-coupled receptor TGR5 (also known as GPCR-1) is expressed on immune cells,^[1] only a few reports study this protein in an inflam-

mation context.^[2] In the course of a drug discovery program for autoimmune diseases, we found that activation of TGR5 by tauro-lithocholic acid (one of its putative natural ligands) selectively inhibits the secretion of pro-inflammatory cytokines that up-regulate the Th1 pathway (INF- γ , TNF- α , IL-12). In contrast, the production of cytokines that are known to promote differentiation along the Th2 axis (such as IL-10 and IL-4) were unaffected. This led us to hypothesize that TGR5 agonism could represent a novel and selective therapeutic principle to treat diseases that are characterized by an overshooting Th1 cell component (like multiple sclerosis, psoriasis, or type 1 diabetes).

The presentation will focus on one non-steroidal chemical series of TGR5 agonists that was discovered by screening the Novartis compound archive. Using key compounds as examples, the structure–activity relationship in a cAMP stimulation assay will be discussed. Generating cross-reactivity for a rodent orthologue presented a particular challenge, and the structural requirements to achieve potency on both human and mouse TGR5 will be highlighted. Potent compounds decreased the lipopolysaccharide (LPS)-stimulated release of TNF- α and IL-12, but not IL-10 in isolated human monocytes and dendritic cells. The pharmacokinetic profile of a selected compound will be presented as well as the reduction of LPS-induced TNF- α and IL-12 production after p.o. administration in vivo. Compound-treated TGR5^{-/-} mice did not show any effect on these cytokines which confirmed that the response in wild-type mice was indeed TGR5 dependent. These results support the initial hypothesis and highlight the potential benefit of TGR5 agonists for the treatment of Th1-driven autoimmune diseases.

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Discovery of Potent, Selective and In Vivo Active Inhibitors of 11 β -Hydroxysteroid Dehydrogenase Type 1

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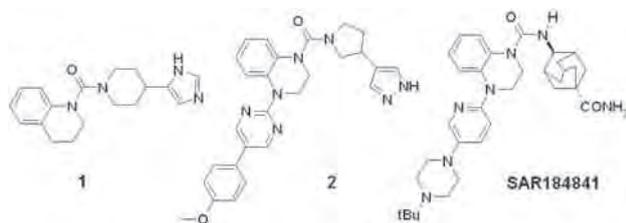
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Glucocorticoid hormones are important chronic regulators of metabolism. Intracellular reactivation of inactive glucocorticoids has emerged as a key mechanism for regulation and amplification of glucocorticoid action. The reactivation is catalyzed by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1). There is evidence implicating an excess of cortisol in tissues as a primary driver of insulin resistance and a critical point for disease intervention.^[1] Liver- or adipose-tissue-specific overexpression of 11 β -HSD1 in transgenic mice produces a phenotype closely resembling human type 2 diabetes mellitus.^[2] Reduction of intracellular corticosterone levels in rodents as a result of pharmacological inhibition of 11 β -HSD1 reverses manifestations of altered metabolic parameters including ectopic fat storage, diabetes, dyslipidemia and atherosclerosis.^[3] These data indicate that inhibitors of 11 β -HSD1 could be novel therapeutics for patients with type 2 diabetes, obesity, and metabolic syndrome.

We have been engaged in a research effort to identify inhibitors of 11 β -HSD1 that are suitable candidates for drug development. A high-throughput screening campaign allowed the identification of a novel class of urea **1** as 11 β -HSD1 inhibitors. Rational chemical optimization provided potent and selective inhibitors **2** of both human and murine 11 β -HSD1 with an appropriate ADME profile and ex vivo activity in target tissues. Final optimization led to SAR184841, which showed good pharmacokinetic parameters and potent activity in pathophysiological animal models. Synthesis, molecular modeling, X-ray analysis, and biological data will be presented.



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Molecular Dynamics Study of the D₂R–mGluR5 Heterodimer in the Inactive State

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G-protein-coupled receptors (GPCRs) are classical molecular targets for antipsychotics. Recently, GPCR heterodimers have attracted particular attention as drug targets for the treatment of schizophrenia. The most important are those involving the D₂R, namely mGluR5–D₂R, A_{2A}R–D₂R, CB₁R–D₂R, NTS₁R–D₂R, and D₂R–D₃R heterodimers. The first evidence for the existence of complexes of glutamate and dopamine D₂ receptors was found in 1984 when it was demonstrated that L-glutamate was able to reduce the affinity of D₂R agonist binding sites. Several years later, it was shown that the mGluR5 is responsible for mediating this antagonistic effect in the mGluR5–D₂R complex. Moreover, combined activation of the A_{2A}R and mGluR5 increased the reduction of the affinity of the D₂R agonist binding sites. Recently, it has been demonstrated that mGluR5, D₂R, and A_{2A}R form higher-order oligomers in living cells. Based on these studies it was proposed that the combined application of low doses of A_{2A}R and/or mGluR5 agonists with or without low doses of D₂R antagonists may be a new strategy for the treatment of schizophrenia.

In light of the above, the aim of this work was investigation of dynamic properties of the mGluR5–D₂R heterodimer. The model of the mGluR5–D₂R heterodimer in the inactive state, bearing the TM5–TM6 interface (see our appropriate abstract) is inserted into a POPC cell

membrane model, and solvated with water and ions. The MD simulations are carried out with GROMACS. The analysis of MD trajectories is performed to assess the stability of the models during molecular dynamics simulation. The RMSD fluctuation is plotted per residue to check which region of protomers or monomers are changed most. To evaluate the differences between the simulation of the respective monomers and protomers in the heterodimer in more detail, essential dynamics analysis is performed. The next step of analysis is devoted to considering the stability and features of the mGluR5–D₂R heterodimer interface. The interface is checked for possible rearrangements during the simulations, and the contacts between the residues forming the interface are monitored. Furthermore, the changes in the conformation of the residues forming the D₂R orthosteric binding site and mGluR5 allosteric binding site are investigated, and the expected effect of dimerization on the binding pocket is evaluated. Additionally, other structural rearrangements likely involved in GPCR function are checked.

Acknowledgements: This study was performed during the post-doctoral stay of A.A.K. at the University of Regensburg, funded by the Deutscher Akademischer Austauschdienst (DAAD). A part of the calculations was performed under a computational grant by the Interdisciplinary Center for Mathematical and Computational Modelling (ICM), Warsaw, Poland, grant number G30-18.

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Hybrid Antibacterials Targeting Fatty Acid Biosynthesis

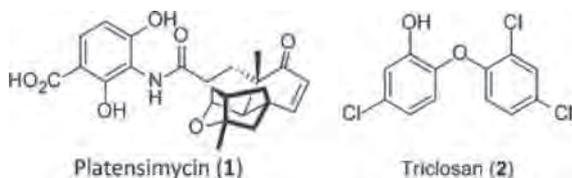
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Rapidly increasing worldwide bacterial resistance to antibiotics has resulted in extensive searches for novel antibacterial agents.^[1] Platensimycin was discovered as a result of a large natural product screening program conducted by Merck in 2006.^[2] Platensimycin (**1**) was described as a potent broad-spectrum antibiotic isolated from strains of *Streptomyces platensis*. A novel mode of action was identified that targets type II fatty acid biosynthesis, specifically binding to the acyl-enzyme intermediate of FabF.

The rising need for effective and novel alternatives to current antibacterial therapies has created great interest toward platensimycin and its derivatives.^[3] Shortly after its discovery, the crystal structure of platensimycin bound to FabF was published in high resolution (2.6 Å), allowing docking studies to be undertaken. We will outline our synthesis of platensimycin derivatives using classical medicinal chemistry in tandem with docking studies. Molecular modelling was used for the identification of key interactions and to evaluate the active site for additional binding pockets. Initial synthetic targets have sought to replace the complex tetracyclic ketolide ring system of platensimycin with simpler substituents that retain the ability to fit and hydrogen bond at the active site. Results to date have shown modest activity for an adamantyl derivative and further experiments are currently underway.^[4]

Furthermore, this project explored the application of designed multiple ligands toward the discovery of novel antibacterial agents by incorporating two prominent pharmacophores into one molecule. Specifically, this project aimed to hybridise the aromatic portion of platensimycin with the widely used antibacterial triclosan (**2**).^[5] As triclosan inhibits elsewhere in the fatty acid biosynthetic pathway (FabI) this strategy will ideally generate inhibitors of both FabF and FabI. Blocking two enzymes in the same pathway may provide advantages with regard to reducing the possibility of bacterial resistance, and refining the pharmacokinetic profile compared to combination therapy.



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P324

Targeted Approaches for Renal Failure

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Laura Badi, Sara Belli, Nicole Kratochwil,
Hans-Jakob Krebs, Helmut Jacobsen, Franz Schuler,
Christophe Schweitzer, Manfred Zell

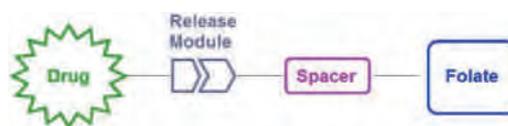
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Targeted therapeutics have increased in prominence offering improved potency and decreased toxicity. There are two main approaches to specifically target an organ: firstly via a receptor expressed specifically on the target organ, or additionally via an enzyme expressed with higher activity in this organ.

Covalent attachment of the vitamin folic acid to almost any small molecule yields a conjugate that can be transported and endocytosed into folate receptor-bearing cells. As folate receptors are significantly overexpressed in kidney and more so in the majority of human cancers, this methodology was used by others for the selective delivery of therapeutic agents to tumor tissue. BMS-753493, a semisynthetic epothilone A folate-prodrug is currently being evaluated for safety

and efficacy in two phase I/II clinical trials in patients with advanced cancer (phase I portion) and advanced ovarian, renal, or breast cancer (phase II portion).^[1]

We pursued a similar strategy for kidney targeting using folate and combining via a spacer, a release module, and a drug which is active on kidney as schematically depicted below. In close analogy to literature precedence^[1] we prepared a key building block from a Roche notch inhibitor and the folate linker which were in turn coupled to form the prodrug.^[2] These folate–drug conjugates were found to be selectively internalized by cells with high levels of folate receptors. The free drug was released intracellularly by the action of sulfhydryl-containing species such as glutathione on the disulfide-containing folate–drug conjugate. After i.v. administration in rats, the kidney/liver selectivity for the notch inhibitor will be described.



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P325

Novel Immunomodulatory Kv1.3 Blockers Based on Diphenoxylate

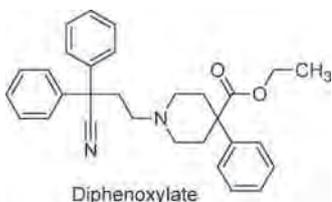
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Autoimmune disorders such as multiple sclerosis (MS) are inadequately treated by current therapies and there is an urgent need for affordable drugs with acceptable safety profiles. The Kv1.3 potassium channel is an exciting new molecular target associated with CCR7⁺ effector memory T (T_{EM}) cells. Autoreactive T cells specific for components of the myelin sheath appear to be crucial for the pathogenesis of MS because of their memory phenotype and their ability to induce experimental autoimmune encephalomyelitis (EAE) in rodents and primates. Selective suppression of these cells has therefore long been an objective for the development of new therapies for MS. Expression of Kv1.3 channels is increased in both CD4⁺ and CD8⁺ T_{EM} cells, and Kv1.3 blockers have been shown to potently inhibit their proliferation without impairing the function of CCR7⁺ naïve and central memory T cells. As such, pharmacological block-

ade of Kv1.3 channels can treat acute and chronic-relapsing animal models of MS without the generalized immunosuppression that occurs with current therapies.^[1]

Previous research has shown that a drug normally used for diarrhea (diphenoxylate, shown) was shown to treat the autoimmune disorder psoriasis;^[2] however, this important observation was not fully evaluated. Our preliminary work has demonstrated that diphenoxylate blocks Kv1.3 channels and may explain the observations that diphenoxylate was able to treat psoriasis. Novel analogues of diphenoxylate were synthesized to explore the SAR of this molecule at Kv1.3 channels. Further synthetic work has built on this initial SAR to generate compounds that are >100 times more potent at Kv1.3 channels with selectivity over Kv1.5 and hERG channels. In addition these potent Kv1.3 blockers are lower in molecular weight and have decreased lipophilicity. These lead compounds represent a new class of Kv1.3 blocker with improved physicochemical properties that have the potential to be developed into CNS agents for MS and other autoimmune disorders.



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P326

Kick-Starting Open Source Drug Discovery for Malaria

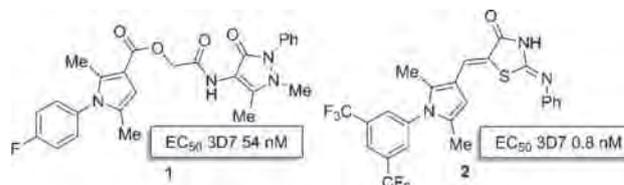
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We recently employed “open source science” to discover an inexpensive resolution of praziquantel (PZQ) to provide a scalable source of the active *R* enantiomer for treatment of schistosomiasis.^[1] We have now turned our attention to *open source drug discovery* for the development of novel antimalarial compounds.

A large database of compounds showing antimalarial activity was published by GSK Tres Cantos in 2010 to act as a starting point in lead identification for drug development.^[2] Our Open Source Drug Discovery Malaria (OSDDmalaria) project will prosecute hit-to-lead campaigns on the most promising series, starting with the arylpyrroles **1** and **2**. We have already developed a range of highly potent compounds (**2**) active against *Plasmodium falciparum* in a whole-parasite assay at picomolar concentrations. The project proceeds quickly through the public contributions of many participants. All of our research is published to our live online lab notebooks and coordination sites.^[3]



By allowing industrial and academic collaborators to identify themselves and participate at any level, we see an acceleration of the research process. Naturally, all the research carried out must be patent-free.

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Free-Energy Calculations for Lead Optimization: How Accurate Can They Be in an Industrial Drug Discovery Context?

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Docking/scoring approaches, which are applied in the early phase of structure-based hit and lead finding, are successful in correctly identifying “good” ligand poses and in enriching ligands against a background of decoy compounds. When it comes to ranking ligands according to binding affinity, the results are less impressive;^[1,2] in particular, they rarely approach “chemical accuracy”, i.e., RMS errors <1 kcal mol⁻¹. This accuracy is needed, however, for successfully guiding a lead optimization campaign in later stages of the drug discovery program.

We studied the performance of three well-established computationally demanding free-energy prediction methods, the molecular mechanics continuum solvent, the linear interaction energy, and the thermodynamic integration approach^[3,4] by using data sets from industrial drug discovery projects: 25 factor Xa inhibitors,^[5] 29 indirubin derivatives inhibiting the cyclin-dependent kinase 2,^[6] and 43 antagonists of the mineralocorticoid receptor.^[7] These data sets cover three different types of target proteins (a serine protease, a protein kinase, and a nuclear receptor) and provide particular challenges, as they contain compounds with highly mobile substituents, different total charges, or diverse structural features. Additional challenges arise from the lack of experimental structural complex information for most of the ligands, unusual protein–ligand interactions, and protein targets with a high inherent mobility. The data sets are, however, typical for an industrial lead optimization setting and should thus allow thoroughly testing the scope and limitations of the free-energy prediction methods.

We found that on these three datasets none of the methods delivers acceptable results with standard settings. However, after target/dataset-specific tweaking, molecular mechanics continuum solvent and thermodynamic integration calculations can yield binding affinity rankings within a time span of two weeks on a state-of-the-art computer cluster that are valuable for lead optimization. We propose a procedure employing a combination of molecular mechanics continuum solvent and thermodynamic integration analyses that allows distinguishing weak and strong binders in heterogeneous ligand sets. The insight into the scope and limitations of binding free-energy calculations gained in this study provides a decision guideline for future method development in the area of more reliable protein affinity prediction.

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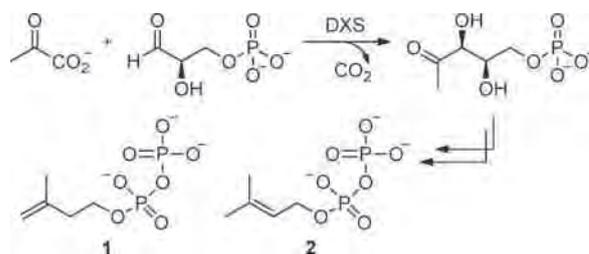
Design, Synthesis and Biological Evaluation of Inhibitors for 1-Deoxy-D-xylulose-5-phosphate Synthase, a Novel Antituberculotic Target

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Tuberculosis (TB) is more prevalent in the world today than at any other time in human history. In recent years, the emergence of several multi- and extensively drug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB, XDR-TB) required the urgent development of new drugs with new modes of action.^[1] *M. tuberculosis*, as well as other pathogens (e.g., *Plasmodium falciparum*) uses the non-mevalonate pathway for the biosynthesis of universal precursors for the essential isoprenoids, isopentenyl diphosphate (IPP, **1**) and dimethylallyl diphosphate (DMAPP, **2**). Given that humans exclusively use the alternative mevalonate pathway, enzymes of the non-mevalonate pathway have emerged as attractive targets for the development of new drugs against bacterial infections like tuberculosis and malaria.^[2]

1-Deoxy-D-xylulose-5-phosphate synthase (DXS) catalyses the first and rate-limiting step of the non-mevalonate pathway (Scheme 1), using thiamine pyrophosphate (TPP) as a cofactor (Figure 1).



Scheme 1. Biosynthesis of IPP (**1**) and DMAPP (**2**) via the non-mevalonate pathway.

DXS was chosen as a target of a structure-based design project. The synthetic genes for 1-deoxy-D-xylulose-5-phosphate synthase from *Deinococcus radiodurans* and *M. tuberculosis* were cloned in pET22 vector and expressed in *Escherichia coli* BL21(DE3) cells. Given the low specific activity at pH 5.0 and 6.0, the inhibition assay was developed at pH 7.6. The dissociation constant for TPP has been determined for *D. radiodurans* DXS (K_d : 114 ± 13 nM).

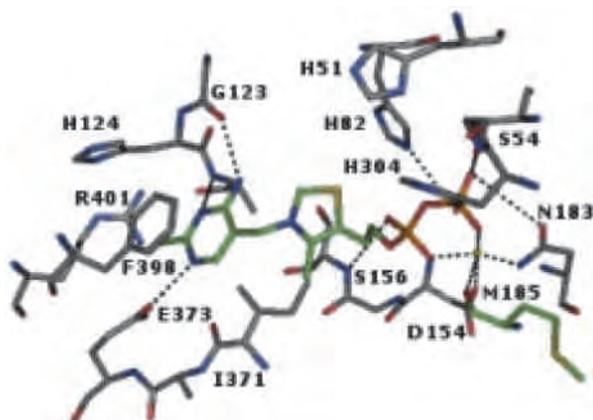


Figure 1. Co-crystal structure of TPP and *D. radiodurans* DXS (3).

The active site of DXS from a model organism (*D. radiodurans*) shows a high degree of homology as well as pathogen-specific features with DXS of *M. tuberculosis*. This has allowed a true de novo structure-based design project in the quest for innovative and selective inhibitors on the way to a new antituberculous drug. Two different scaffolds have been designed and thereafter synthesized to display competitive inhibition with respect to TPP. Several fragments have also been synthesized in order to validate the predicted binding mode.

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P329

8-Phenylethynylxanthines—Highly Potent and Selective Adenosine A_{2A} Receptor Antagonists

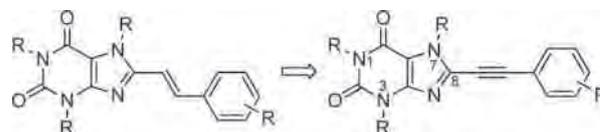
Christa E. Müller, Jörg Hockemeyer, Nikolay Tzvetkov, Hamid Radjainia, Amelie Zech, Petra Küppers, Simone Siebers, Judith Paschkowiak, Meryem Köse

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Adenosine A_{2A} receptor antagonists have been demonstrated to be effective in animal models of Parkinson's disease, and several A_{2A} antagonists are currently undergoing clinical evaluation.^[1] They not only show positive effects on motor symptoms without causing dyskinesia, but have also been shown to mediate neuroprotective effects, and therefore may be true disease-modifying agents suitable for the treatment of neurodegenerative diseases in general, including Alzheimer's and Parkinson's disease.^[2] The first class of nonselective adenosine receptor antagonists with moderate affinity were the natural xanthine derivatives theophylline and caffeine. The introduction of a styryl group at the C8 position of xanthines

was essential for obtaining compounds with enhanced A_{2A} receptor affinity and selectivity by decreasing A₁ affinity. However, the presence of the double bond at the 8-position in 8-styrylxanthines led to photosensitive compounds.^[3] Replacement of the styryl double bond by a triple bond yielded the photostable 8-phenylethynylxanthines.^[5]

To study the structure–activity relationships of this new class of A_{2A}-selective antagonists, we introduced a variety of substituents at different positions, in particular at the ring nitrogen atoms N1, N3, and N7, as well as differently substituted phenylethynyl residues at C8. Thus we obtained derivatives showing high affinity at the A_{2A} receptor in the low nanomolar range combined with excellent selectivity. One of the most potent derivatives, 3-cyclopropyl-8-(3,4-dimethoxyphenylethynyl)-7-methyl-1-(2-propynyl)xanthine, was obtained in tritium-labeled form ([³H]PSB-1010) from its 7-demethyl precursor, and is used as a specific A_{2A} radioligand.



Acknowledgements: Support by the BMBF (BioPharma Neuroallianz) is gratefully acknowledged.

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P330

Search for Common Pharmacophore Patterns of TRPV1 Antagonists

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Transient receptor potential vanilloid type 1 (TRPV1) plays a major role in pain perception in humans. Therefore, discovery of its ligands as a new class of non-opioid analgesics for the treatment of chronic pain is an area of intense research. Because agonists of the receptor cause a highly undesirable side effect of initial sharp pain sensation, most of the ongoing studies focus on the development of TRPV1 antagonists, using the structures of previously tested compounds.^[1]

This study was aimed at finding a pharmacophore pattern shared by most of the active antagonists of the vanilloid receptor, taking into consideration their extremely broad chemical space. Out of a data set of 607 TRPV1 antagonists compiled from the literature, five compounds with high potency values (IC₅₀ < 10 nM) and similar shape and size were selected. Pharmacophore modeling was performed in LigandScout,^[2] and the final model (Figure 1) contained six features,

but did not contain an H-bond donor as was previously predicted.^[3] Validation of the model by virtual screening of the available set of TRPV1 ligands led to a model with a global accuracy of 0.6. After ranking compounds according to the pharmacophore fit score, 62.8% of initial true positive hits (TP) and only 28.8% of initial false positive hits (FP) were among the top-ranked compounds.

The model was also validated for prediction of a set of 18 compounds undergoing clinical studies,^[3,4] whereby eight ligands were found as top-ranked hits. This model thus represents a versatile tool for prediction of new chemical scaffolds for TRPV1 antagonists.

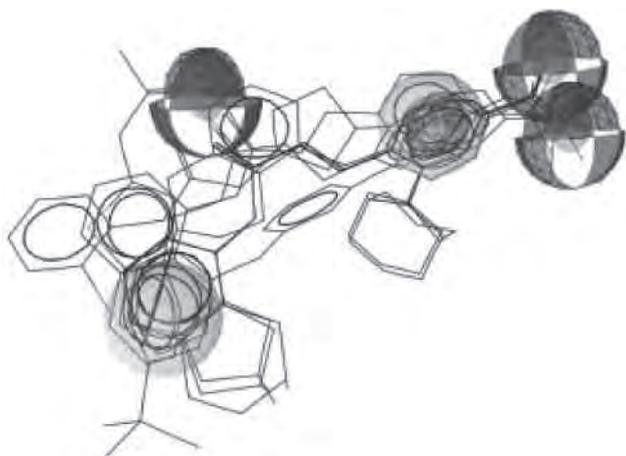


Figure 1. Superposition of the eight top-ranked hits to the pharmacophore model. Light-grey denotes hydrophobic features, grey abrupt regions to represent H-bond acceptor atoms in the ligands, and the darker ring to indicate the aromatic moiety.

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Fluoro-Keto-Pyrrolyl Derivatives as Aldose Reductase Inhibitors

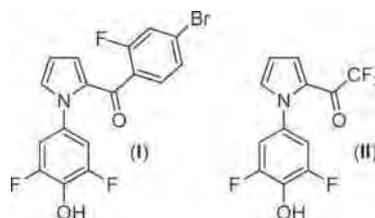
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In recent years, a striking increase in the cases of diabetes mellitus has been observed worldwide, tending toward epidemic prevalence. Aldose reductase (ALR2, AR, AKR1B1, EC 1.1.1.21) belongs to the aldo-keto reductase superfamily. It is the first enzyme of the polyol pathway, which converts glucose to sorbitol by using NADPH as a cofactor. The second (and last) enzyme of the pathway is sorbitol dehydrogenase (SDH), which converts sorbitol to fructose, using NAD⁺ as a cofactor. The physiological role of ALR2 is detoxifying and regulating, but in cases of diabetes and/or hyperglycemia, glucose is converted rapidly to sorbitol, which tends to concentrate in the cells, damaging them in many tissues. Therefore, ALR2 was initially found to be responsible for the long-term complications of diabetes such as neuropathy, nephropathy, retinopathy, and cataracts.

However, a number of reports have suggested that under normal glucose concentrations, ALR2 could be up-regulated due to factors other than hyperglycemia. This implies that the enzyme is additionally responsible for pathological states, such as cardiovascular disorders, mood disorders, inflammation, renal insufficiency and ovarian abnormalities. Furthermore, ALR2 is found to be overexpressed in some particular types of human cancers. These new findings have drawn even more the attention of the scientific community toward finding new, efficient and, safer aldose reductase inhibitors, as currently only one is on the market.

In our search for novel ARI chemotypes,^[1] we have prepared and tested in vitro a number of aroyl-pyrrolyl-difluorophenol derivatives. The synthetic strategy involved an efficient pyrrole ring formation under Clauson–Kaas cyclization conditions, catalyzed with nicotinamide, as well as a regioselective Friedel–Crafts arylation in the presence of a defined ratio of AlCl₃/aroyl chloride. We found that the most active derivative was the (4-bromo-2-fluorophenyl) (1-(3,5-difluoro-4-hydroxyphenyl)-1H-pyrrol-2-yl)methanone (**I**) with an ALR2 inhibitory IC₅₀ value of 190 nM. We consider this compound a promising lead, derived from the hit scaffold of pyrrolyl-difluorophenol ARIs. It was also noted that the presence of an aroyl moiety is not a prerequisite for activity. For example, 1-(1-(3,5-difluoro-4-hydroxyphenyl)-1H-pyrrol-2-yl)-2,2,2-trifluoroethanone (**II**) exhibited an inhibitory IC₅₀ value of 930 nM.



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P332

In Vitro and In Vivo Characterization of Novel Inhibitors of Toxic β -Amyloid Aggregation

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, affecting nearly 25 million patients. It is characterized by progressive cognitive decline and eventually debilitating dementia. Currently available pharmacologic interventions only provide symptomatic relief without halting the progression of the disease. Thus, there is an enormous medical need for novel disease-modifying therapies that target the underlying neuropathological mechanisms involved in the development of AD. Strong genetic, physiological, and biochemical evidence suggests that β -amyloid ($A\beta$) plays a key role in AD. Preventing $A\beta$ aggregation is therapeutically attractive, because this process is believed to be the main pathological event, and does not interfere with the physiological role of the amyloid precursor protein (APP).

We have employed a set of rationally designed non-dye compounds. The aim was to inhibit $A\beta$ 1–42 oligomerization and to disassemble pre-formed $A\beta$ 1–42 oligomers. The ThT assay, used as an initial screening tool, was complemented by other biochemical assays and allowed the identification of compounds with suitable inhibition of $A\beta$ 1–42 aggregation. Several optimization rounds allowed the discovery of a sub-series with enhanced metabolic stability while maintaining other key pharmacological properties. By using an in vitro cell-based assay, the capacity of our compounds to rescue PC12 neuronal cells from $A\beta$ 1–42-mediated toxicity was also investigated. Additional ADME-Tox evaluation enabled the selection of three compounds with suitable brain penetration. These compounds were then tested in a female hAPPL transgenic mouse model using behavioral and biochemical readouts.

We have discovered a set of small molecules that prevented/reversed the pathological toxic effect of $A\beta$ and improved memory deficits of female hAPPL mice. Thus, these compounds could be promising candidates for the treatment of neurodegeneration in AD and related amyloid diseases.

P333

Discovery of Inhibitors of Bacterial Enzyme D-Aspartate Ligase

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A well validated target for antibacterial therapy is the system of enzymes responsible for the construction of peptidoglycan, an essential component of the bacterial cell wall that provides the structural integrity that is necessary for bacterial cells to resist internal osmotic pressure.^[1] The bacterial enzyme Asl_{fm} , the D-aspartate ligase of *Enterococcus faecium*, is a member of the ATP-grasp protein superfamily and catalyzes the ATP-dependent carboxylate–amine ligation reaction, and appears to be a new attractive target for the development of narrow-spectrum antibacterials active against multidrug-resistant *E. faecium*.^[2]

Asl_{fm} is an ATP-dependent enzyme. Recent studies demonstrated the feasibility of finding ATP-competitive antibacterials with good selectivity profiles and indicate that the ATP-binding site can be a promising target for antibacterial drug design.^[3] Because a crystal structure of the Asl_{fm} enzyme is not known yet and the substrate binding site is large, we focused on the ATP-binding site and screened a small collection of ATP-competitive inhibitors of ATP-grasp enzymes. This approach resulted in first known inhibitors of Asl_{fm} . Selected inhibitors were additionally used as a starting point for ligand-based drug design. Pharmacophore modeling was performed using LigandScout, a ligand-based pharmacophore generator.^[4] The pharmacophore model with the highest score was used in the large-scale virtual screening campaign by screening the library of approximately 5.5 million commercially available compounds. After visual inspection, a total of 14 compounds from the virtual screen were selected for testing of their in vitro inhibitory activities on *E. faecium* D-aspartate ligase by pyruvate kinase/lactate dehydrogenase-coupled enzymatic assay.

A series of low-micromolar-weight Asl_{fm} inhibitors, based on the pyrazolo[3,4-*d*]pyrimidine scaffold and targeting ATP-binding site, was discovered. Inhibitors will be tested against *E. faecium*, and additional enzyme structure studies will enable further structure-based optimization of compounds.

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P334

Platinum Complexes of Guanidinium-Based DNA Minor-Groove Binders: Conjugating Forces towards Better Anticancer Agents

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Since 1971, the *cis*-dichlorodiamino platinum(II) complex cisplatin (Figure 1) has been found to be efficacious in treating various tumours, especially those in testicular and ovarian cancer. However, this complex suffers from a number of drawbacks, most noticeably severe nephro- and ototoxicity and acquired and intrinsic resistance to certain cancers.^[1] At the moment there are only two other platinum-based chemotherapies licenced for clinical use worldwide: carboplatin, a less toxic cisplatin derivative, and oxaliplatin, which is used clinically in treating colon cancer (Figure 1).^[2] A number of platinum-based cancer therapies are currently in clinical trials.^[3] Platinum-based dual drugs may overcome the resistance and toxicity problems and may broaden the range of cancer targets that are currently being investigated to treat cancer. Marmion's Pt^{II} histone deacetylase (HDAC) inhibitor has shown promising results in cell lines susceptible and resistant to cisplatin treatment.^[4] Based on this, we proposed that the diaromatic bis-guanidinium-like DNA minor groove binders (MGBs) prepared in our laboratory^[5] could be functionalised without loss of DNA binding affinity to create cisplatin analogues with synergistic antitumour effects. These MGBs, acting as delivery vehicles guiding the diamminoplatin moiety to the nucleus, may aid in decreasing side effects and increase the concentration of cisplatin's active form around DNA.

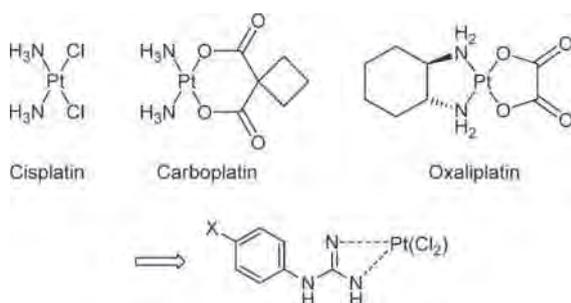


Figure 1. Structures of clinically used Pt^{II} complexes and generic structure of some of the compounds presented herein.

We have now carried out computational docking experiments on existing and proposed MGB motifs which have confirmed that the proposed drug candidate binds strongly to the minor groove (Figure 2). Additionally, work is on-going to prepare Pt^{II} complexes by complexation with the guanidinium moiety of different derivatives.

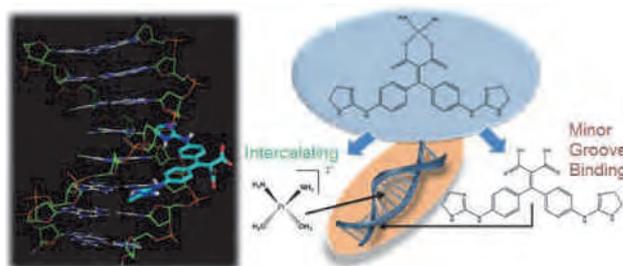


Figure 2. Docking of the MGB moiety of our proposed Pt^{II} complex.

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P335

Synthesis and Anti-inflammatory Activity of 5-Nonsubstituted/Substituted 2-[(4-Adamantinethiazol-2-yl)imino]-4-thiazolidinones

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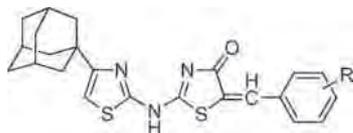
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Inflammation is a physical response of the human organism to tissue damage caused either by injuries or by pathogens, chemical substances and other irritant factors. Chronic inflammation is considered to be involved in a host of diseases, including atherosclerosis, rheumatoid arthritis, asthma and even neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases.

Thiazolidinones have received considerable attention due to their wide range of pharmacological action, as they are known to possess anti-inflammatory activity, for example. Adamantane is also well known for its potential as a biologically active nucleus. Thus, in our outgoing project, we moved forward with our investigation by linking thiazole and adamantane to a thiazolidinone ring, synthesizing, in a three-step reaction, twelve new compounds of the general structure shown (see figure).

To investigate the anti-inflammatory activity, the carrageenan-induced mouse paw edema in vivo method as well as in vitro inhibition of COX-1/COX-2 enzymes, which are considered to be involved in inflammation, were performed. Most of the synthesized compounds showed very good anti-inflammatory activity and in some cases even excellent; therefore, they are considered promising anti-inflammatory agents.



P336

Optimization and Pharmacological Evaluation of Imidazo[1,2-*a*]pyridine-Based Inhibitors of 5-Lipoxygenase

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Leukotrienes (LT) are lipid mediators of immune and inflammation responses, with important roles in respiratory (asthma), cardiovascular diseases (atherosclerosis),^[1] and certain types of cancer.^[2] LT are formed from arachidonic acid (AA), which is released from membrane phospholipids by phospholipase A₂ (cPLA₂), and it is metabolized by 5-lipoxygenase (5-LO) in the nuclear membrane.

5-LO is a dioxygenase that catalyzes the incorporation of both atoms of molecular oxygen into AA in two steps to give the hydroperoxide 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HpETE) as well as the following dehydration to the unstable epoxide LTA₄. Subsequent conversion of LTA₄ by LTA₄ hydrolase leads to leukotriene B₄ (LTB₄), and the conjugation with glutathione (GSH) by LTC₄ synthase yields the cysteinyl leukotriene C₄ (LTC₄). In addition to the conversion into LTA₄, 5-HpETE can be reduced to the resultant alcohol 5-HETE.

In the inactive form of the enzyme, the iron Fe²⁺ (ferrous) and the enzyme is not capable of converting AA to the corresponding LTs. Fe²⁺ has to be oxidized by lipid hydroperoxides (LOOH) to the active Fe³⁺ to perform the catalytic cycle.^[3] (±)-1-(1-Benzo[*b*]thien-2-ylethyl)-1-hydroxyurea, also called zileuton (Zyflo®), was the first 5-LO inhibitor^[4] that entered the US market in 1997 for the chronic treatment and prophylaxis of various clinical phenotypes of asthma. Nevertheless, zileuton exhibits liver toxicity^[5] that is unrelated to the inhibition of 5-LO, thus its clinical use is limited.

Consequently, there is a need for the design and development of new direct inhibitors of 5-LO that exhibit high efficacy, irrespective the mode of activation. Potential drug candidates should exhibit high efficacy in vitro and in vivo, as well as desired pharmacological properties such as low hepatotoxicity and good oral bioavailability. In the present study we describe the optimization of our lead compound *N*-

cyclohexyl-6-methyl-2-(4-morpholinophenyl)imidazo[1,2-*a*]pyridin-3-amine (EP6), pharmacologically evaluated,^[6,7] which derived from a virtual screening for cyclooxygenase (COX)/5-LO dual inhibitors.^[8] EP6 is a novel direct 5-LO inhibitor independent of the stimulus of enzyme activation or cellular redox state, it interacts with the regulatory C2-like domain.

Imidazo[1,2-*a*]pyridine can be easily obtained by means of the Groebke–Blackburn–Bienaymé^[9] multi-component reaction (MCR). In this study we present the synthesis and pharmacological optimization of a series of *N*-fused imidazoles with improved activity and pharmacological and physicochemical properties: safety, efficacy in vitro and in vivo, and metabolism.

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P337

Development of Dual Inhibitors of 5-Lipoxygenase and Soluble Epoxide Hydrolase

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Over the decades, the “one drug—one target—one disease” approach was the predominant principle in drug development. However, when considering treatments of complex diseases, interference with one target may be not enough. Clinical studies on approved drugs showed that we may consider a rational design of drugs that interfere with multiple targets, but do not interact with off-targets responsible for side effects.^[1,2] Our project involves the development of novel ligands that inhibit two enzymes of the arachidonic acid cascade: 5-lipoxygenase (5-LO) and soluble epoxide hydrolase (sEH). 5-LO catalyzes two steps in the biosynthesis of leukotrienes, which regulate the innate immune response and play a pathophysiological role in chronic inflammatory diseases such as asthma and

atherosclerosis.^[3] sEH mediates the hydrolysis of epoxyeicosatrienoic acids (EETs) to their corresponding diols, the dihydroxyeicosatrienoic acids (DHETs).^[4] EETs generally induce anti-inflammatory effects and have been reported to exert beneficial effects in diseases such as hypertension, diabetes, stroke, dyslipidemia, pain, immunological disorders, eye diseases and other indications.^[5] Both sEH^{-/-} mice and animals treated with sEH inhibitors show an increased level of EETs and 5-LO products, indicating that sEH inhibitors seem to synergize with COX and 5-LO inhibitors. Co-administration of the sEH inhibitor *t*-AUCB with either a COX or 5-LO inhibitor showed a significant enhancement of their anti-inflammatory activities.^[6] sEH inhibitors alone induced albumin urea in mice, probably due to a shift toward the 5-LO branch of the arachidonic acid cascade.^[7] Therefore, the development of dual sEH/5-LO inhibitors might lead not only to highly interesting and effective anti-inflammatory compounds, but also to safer anti-hypertensive drugs. The first approach in our project toward the development of a dual inhibitor was the synthesis of hybrid molecules combining one pharmacophore for each target via linkage. As 5-LO inhibitor, we used the recently published imidazo[1,2-*a*]pyridine EP6.^[8] Urea compounds are a well-known class of sEH inhibitors and are therefore used in our synthesis as the other pharmacophore. We synthesized a small library of dual ligands, which showed good inhibitory potential toward both enzymes *in vitro*.

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P338

A Prenylchalcone Found in Hops is a Highly Potent Inducer of Differentiation in Neuronal Stem Cells

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Diseases like Alzheimer's or Parkinson's as well as ischemic incidences are all related to a loss of neuronal tissue. Regeneration of the destroyed cell population would lead to an improvement for all

patients with neurodegenerative diseases. In the last decade the plasticity of human brain was unveiled and related to the presence of stem cells, also in the adult human brain. These neuronal stem cells can differentiate into neurons (neurogenesis) or microglia such as oligodendrocytes and astrocytes (gliogenesis). A selective induction of neurogenesis can be the first step toward a possible regeneration strategy of destroyed neuron populations.

Hops, *Humulus lupulus L.*, mostly associated with the brewing industry, contain a special class of flavonoids: the prenylflavonoids. All prenylflavonoids have prenyl, geranyl, farnesyl, lavandulyl groups or pyrano or furano rings in common. An advantage of prenylflavonoids is their higher lipophilicity induced by their prenyl side chain.^[1] Moreover, some studies have provided evidence that prenylflavonoids can cross the blood–brain barrier.^[2,3] This study addressed the question whether rare prenylflavonoids from hops can promote neuronal differentiation in stem cells.

We identified a prenylchalcone of hops as a potent and special inducer of neurogenesis. By using a luciferase–reporter gene assay based on double cortin, the activity of prenylflavonoids in neurogenesis induction was determined. With this assay on hand, we fractionated a hop extract, which is enriched in prenylflavonoids, based on their activity. We identified prenylchalcones in a low polar fraction as very potent inducers of neurogenesis with even higher activity than the known differentiation factor retinoic acid and other known flavonoids. In contrast to retinoic acid, no differentiation to oligodendrocytes was induced. The structural characteristics responsible for this induction of neurogenesis were revealed by some closely related derivatives. Synthesis of flavanones also containing this neurogenesis-inducing characteristic also led to a higher activity in induction of neurogenesis relative to starting compounds.

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P339

Design, Synthesis and Biological Evaluation of Small Benzylidene Derivatives as Selective Inhibitors of the Protein Kinase DYRK1A

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The dual specificity tyrosine phosphorylated and regulated kinase 1A (DYRK1A) is a member of the GMGC family of protein kinases and plays a vital role in cellular mechanisms such as regulation of transcription, mRNA splicing, and neurodegeneration. The human DYRK1A gene is located in a specific zone of the genome called

“Down syndrome critical region” (DSCR) on chromosome 21. Its overexpression in Down syndrome (DS) is suggested to contribute to developmental brain defects and the early onset neurodegeneration in individuals with trisomy 21. In particular, phosphorylation of the microtubule-associated tau protein by DYRK1A suggests the involvement of DYRK1A in neurofibrillary degeneration in DS. The specific inhibition of this kinase is therefore important in order to abrogate the effects of its overexpression. In this study, we present a series of benzylidene compounds as novel inhibitors of DYRK1A. Especially because of the small size of the molecules (M_r <300 Da), selectivity toward most other kinases is expected.

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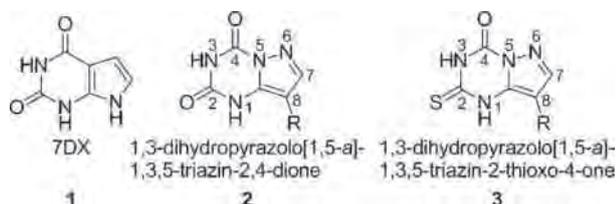
P340

Derivatives of Pyrazolo[1,5-*a*][1,3,5]triazines as Inhibitors of Thymidine Phosphorylase

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Thymidine phosphorylase (TP) is an enzyme involved in tumor angiogenesis. It can also catalyze the phosphorolysis of some nucleotide drugs, thereby decreasing their bioavailability. Therefore, inhibitors of TP may possess therapeutic value in the treatment of cancer. Currently, only one TP inhibitor has entered clinical trials, and it is worthwhile to develop more inhibitors as potential drug candidates. It has been reported that purine analogue 7DX (**1**) exhibits TP inhibitory activity; therefore, we hypothesize that the pyrazolo[1,5-*a*]-[1,3,5]triazine scaffold, which is recognized as the bioisostere of purine, may possess TP inhibitory activity when appropriately substituted. In this study, a series of compounds derived from the pyrazolo[1,5-*a*][1,3,5]triazine scaffold was synthesized and investigated for inhibition against TP as a preliminary study to develop new TP inhibitors.



The synthesis of the target compounds was carried out in a two-step reaction. Key intermediates, namely *N*-ethoxycarbonyl-*N'*-(pyrazol-3-yl)ureas for 2,4-diones (**2**) and *N*-ethoxycarbonyl-*N'*-

(pyrazol-3-yl)thioureas for 2-thioxo-4-ones (**3**), were prepared via addition of ethoxycarbonyl isocyanate or ethoxycarbonyl isothiocyanate, respectively, to variously substituted 3-aminopyrazoles. These intermediates were then subjected to ring annulation reactions to generate the target compounds. Inhibitory activity of these compounds against recombinant human TP was evaluated by a continuous UV spectrophotometric enzyme assay using thymidine as the substrate, whose decrease in absorbance was monitored at λ 290 nm.

A total of 34 compounds with different substitutions at position 8 of the pyrazolo[1,5-*a*][1,3,5]triazine scaffold were successfully synthesized (yields 43–94 %). Based on the results of the *in vitro* TP enzyme assay, it was found that 1,3-dihydropyrazolo[1,5-*a*][1,3,5]triazin-2-thioxo-4-ones (**3**) were more potent against TP than their 2,4-dione analogues (**2**). In addition, compounds with substituted phenyl groups exhibited better activity than those without the phenyl ring, while the more hydrophobic and electron-withdrawing substituents would give more potent compounds. The best compound showed an IC_{50} value <0.1 μ M, and it was more potent than the lead compound 7DX (IC_{50} : 32 μ M under the same evaluation conditions).

The hypothesis that suitably substituted pyrazolo[1,5-*a*][1,3,5]triazines will possess TP inhibitory activity was found to be true. Bioisosteric substitution of oxygen with sulfur at position 2 and attaching substituted phenyl groups at position 8 are necessary for inhibitory activity against TP. Our goal to develop new TP inhibitors was fulfilled, and further biological work would elucidate further mechanisms of action.

P341

Synthesis and Evaluation of Substituted Chroman-4-one and Chromone Derivatives as Sirt2-Selective Inhibitors

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Sirtuins (SIRT2) are enzymes which are highly conserved from bacteria to humans. They function as deacetylating enzymes on lysine residues of various histones and non-histone substrates with the special requirement of nicotinamide adenine dinucleotide (NAD⁺).^[1] This enzyme class is attractive as drug targets as they are involved in important cellular processes such as aging, and hence in neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's disease.^[2] Sirt2 in particular is involved in cell cycle regulation, and its inhibition leads to hyperacetylation of α -tubulin, which relates the enzyme to cancer. Therefore, Sirt2-selective inhibitors are of great interest.

Our group has a long-term interest in chromone and chroman-4-one derivatives. We have successfully developed methods for the synthesis of 2-alkyl-substituted chroman-4-one and chromone-based

scaffolds using a base-promoted aldol condensation/intramolecular Michael addition reaction.^[3] In the course of our work we could identify a 2-pentyl-substituted chroman-4-one derivative as a selective Sirt2 inhibitor with an IC₅₀ value of 4.5 μM. A set of chroman-4-ones based on this lead compound has been synthesized to explore the structure–activity relationships. In addition we are studying additional bicyclic derivatives based on other scaffolds as selective Sirt2 inhibitors.

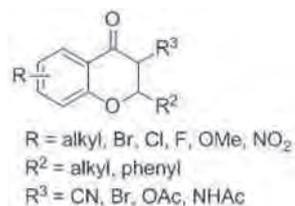


Figure 1. Substituted chroman-4-ones as selective Sirt2 inhibitors.

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P342

DC-SIGN Antagonists: a Paradigm of C-Type Lectin Binding Inhibition

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Carbohydrates have long been underappreciated by the scientific community, but have recently gained much publicity.^[1] Cell-surface carbohydrates named glycans allow intercellular communication by binding to the carbohydrate-binding proteins (CBPs). Many pathophysiological processes like pathogen–cell contact rely upon these interactions. At the moment, more than 80 CBPs have been identified.^[2] However, only few of them have been thoroughly studied, and as a result, few CBPs have been recognized as drug targets. DC-SIGN is a C-type lectin on dendritic cells that binds invading pathogens and mediates an adaptive immune response from T cells.^[3] Additionally, DC-SIGN serves as a signaling receptor that mediates DC maturation and the intensity of the adaptive immune response.^[4] Some pathogens take advantage of this mechanism as they deter DC maturation through DC-SIGN-mediated signaling and inhibit antigen presentation to T cells. HIV-1 enters DC via DC-SIGN and avoids lytic degradation. Thus, HIV-1 not only escapes the host immune system but is presented directly to CD4⁺ T cells, which enables fully

disseminated HIV-1 infection.^[5] Inhibition of pathogen interaction with DC-SIGN-specific antagonists is considered a plausible concept for new anti-HIV agents.^[2]

DC-SIGN specifically binds mannose and fucose-glycosylated endogenous proteins (ICAM-2 and -3) as well as mannosylated PAMPs (HIV-1 gp120).^[2] Moreover, D-Man and L-Fuc-containing oligosaccharides bind to DC-SIGN with moderate to high affinity.^[6] As a consequence, glycomimetic structures designed to bind DC-SIGN have been based on oligomannosides or on Lewis-x.^[2,7]

We have designed, synthesized and assayed novel DC-SIGN antagonists using a glycomimetic approach. Reported aa-fucosylamides bind to DC-SIGN with high micromolar inhibitory constants,^[8] and pseudo-1,2-mannobioside with moderate antiviral activity in the Ebola infection model (IC₅₀: 0.62 mM), while its azide derivative, inhibited DC-SIGN adhesion with IC₅₀: 1.1 mM (measured by SPR).^[9] Moreover, tetravalent dendron-containing four copies of a linear trimannoside mimic inhibits the trans-HIV infection process of CD4⁺ T lymphocytes in the low micromolar range.^[10] To improve binding affinities of monovalent glycoconjugates, we have designed glycoconjugates based on pseudo-1,2-mannobioside that could bind into hydrophobic binding pockets on DC-SIGN CRD unoccupied by native ligands.^[11] These binding pockets were identified by careful examination of crystal structure of DC-SIGN CRD in complex with the tetramannoside Man₄. We synthesized promising candidates and determined their affinities to DC-SIGN by an in vitro assay that measures inhibition of DC-SIGN-mediated immature dendritic cell adhesion.^[12] We also performed docking studies to rationalize the results and to suggest further optimization. The assay data demonstrate that our efforts to design and synthesize mannose-based DC-SIGN inhibitors resulted in compounds that inhibit DC-SIGN-mediated adhesion in the low micromolar range. Ongoing research is directed toward high-affinity monovalent DC-SIGN ligands that will be conjugated onto oligovalent supports. The concept of oligovalent conjugates will be presented as a plausible way to tackle not only DC-SIGN, but C-type lectin binding inhibition in general.

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P343

Studies on the Molecular Basis for VPg Inhibition by FUTP in Foot-and-Mouth Disease Virus

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Foot-and-mouth disease (FMD) is a highly contagious disease that affects cloven-hoofed animals, including domestic and wild bovids. The virus responsible for the disease is foot-and-mouth disease virus (FMDV), which belongs to the picornavirus family. These kinds of viruses use a protein of 20–24 residues, termed viral protein genome-linked (VPg), to initiate viral RNA synthesis. During replication initiation, the first step is the linkage of a UMP unit to the Tyr3 hydroxy group of the VPg protein. Thus, virally encoded RNA-dependent RNA polymerase (3D) requires the uridilylated form of VPg to act as the primer for both positive- and negative-strand synthesis.

Recent studies in FMDV^[1] showed that 5-fluorouridine triphosphate (FUTP) may act as a potent competitive inhibitor of VPg uridylation. In this way, peptide analysis by mass spectrometry has identified a VPg fragment containing FUMP covalently attached to Tyr, but the molecular basis of this block is still unknown.

To investigate this possible novel role for FUMP, the synthesis and X-ray studies of two models of VPg1 that contain U or FU in a 15-mer peptide linked through the hydroxy group of Tyr3 we will be presented. Interestingly, an X-ray co-crystal structure of 3D-pol FMDV/VPg-FU showed a significant conformational change at the $\beta 9$ – $\alpha 11$ loop, protruding into the active site of the polymerase, thus blocking access of the template and of the incoming nucleotides.

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P344

Design of Bitopic Ligands for G-Protein-Coupled Receptors

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Recently dualsteric (bitopic allosteric/orthosteric) ligands have been added to the drug discovery arsenal. The strategy is derived from the message–address concept introduced by Schwyzer in the late 1970s. Dualsteric ligands bind simultaneously to the orthosteric and allosteric binding sites of a receptor protein. The orthosteric binding site of the endogenous activator is typically highly conserved among the subtypes of a G-protein-coupled receptor. In contrast, the allosteric site of the same receptor, which is often located in the vestibule of the orthosteric site, is far less conserved. An optimal bitopic ligand consists of a high-affinity agonistic or antagonistic moiety binding to the orthosteric pocket (the message) and a highly subtype-selective allosteric (the address) docking to the allosteric vestibule of the receptor, both fragments being connected via an optimized linker. Thus, the concept of dualsteric ligands is an avenue to subtype selectivity, and furthermore, signaling pathway selectivity. This will be demonstrated for dualsteric agonists and antagonists of muscarinic receptors, and the binding mode of the ligands (Figure 1) will be discussed.

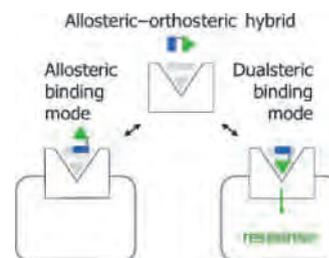


Figure 1. Modes of bitopic ligand binding (blue: allosteric antagonist moiety; green: orthosteric agonist moiety).

P345

3-Trifluoromethylquinoxaline 1,4-Di-*N*-oxide Derivatives as Anti-trypanosomatid Agents. Synthesis, Biological Evaluation and Electrochemical Studies

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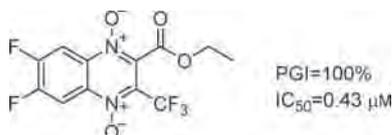
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Chagas disease, or American trypanosomiasis, caused by the protozoan *Trypanosoma cruzi*, is the largest parasitic disease burden in the Latin American countries. It affects approximately eight million people. Every year, 12500 people die from this parasitosis, and over 42000 new cases arise.^[1] Currently, there are only two clinically used drugs: Nifurtimox and Benznidazole. Both possess significant toxic effects and relative clinical efficacy;^[2] therefore, the pharmacotherapy of Chagas disease is very deficient, and there is an urgent need for the development of safe and effective drugs.

Based on the demonstrated anti-infective capability of the quinoxaline system against a large number of microorganisms, our group evaluated a selected group of derivatives. This study allowed us to identify excellent in vitro anti-*T. cruzi* agents and to state the correct requirements for obtaining optimal in vitro anti-*T. cruzi* activity. The *N*-oxide moiety seems to be essential for anti-*T. cruzi* activity, and derivatives with electron-withdrawing substituents at the 2-, 3-, 6-, and 7-positions of the quinoxaline ring were the most active compounds.^[3,4] Moreover, some derivatives have appeared as mitochondrial dehydrogenase inhibitors.^[3]



In this work we present the synthesis and biological evaluation against *T. cruzi* of new 3-trifluoromethylquinoxaline 1,4-di-*N*-oxide derivatives. Moreover, the reduction potential of these derivatives has also been studied with the aim of establishing the substituents which could facilitate reduction of the *N*-oxide, leading to more active compounds.

Taking into account the promising results of these compounds, mutagenicity assays have also been performed and in vivo assays are being performed with the most promising derivatives.

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P346

A-, B- and D-Ring-Modified Steroidal Aromatase Inhibitors: Design, Synthesis and Biochemical Studies

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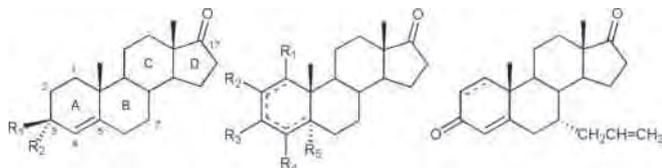
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Aromatase, a cytochrome P450 enzyme, is involved in the last step of the biosynthesis of estrogen, and is a key factor in the promotion of hormone-dependent breast cancer growth. Aromatase inhibitors are now a standard of care in the management of hormone-responsive early breast cancer in postmenopausal women. Targeting aromatase is the basis of a highly selective treatment; however, two major issues continue to arise: the adverse effects such as decrease in bone mineral density and joint symptoms, as well as the recently described emergence of tumor cell resistance.^[1]

In this work we present the results of the biological evaluation of three sets of A-, B- and D-ring-modified androstenedione-based inhibitors, most of them designed and synthesized in our laboratory. One of these sets includes the C3 hydroxy derivatives, which were found to be very active compounds, especially if the C3 hydroxy group assumes a 3 β -stereochemistry. In another set of compounds we studied the influence of double bonds or epoxide functions along the A-ring, as it is known that planarity and probably some electronic density in this ring, as well as in the A/B-ring junction, are important for aromatase inhibition.^[2] It was observed that olefins are better aromatase inhibitors than epoxides, except for the 3,4-epoxide/olefin pair, in which the epoxide is the better inhibitor. This reveals the possibility of the 3,4-oxirane oxygen to resemble the C3 carbonyl group of androstenedione, the natural substrate of the enzyme, allowing

the establishment of a hydrogen bond with a receptor residue.^[3] It has also been reported that the 7 α -substitution of androstenedione allows enhancement of the affinity toward aromatase.^[4] Therefore, we embarked on the preparation of another set of 7 α -allyl derivative compounds, with some A-ring key features also being explored, revealing strong aromatase inhibitors. The results of this study will be presented in this communication.



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P347

Lipase Inhibitors Mimicking the Substrate: Isolation and Synthesis of Their Derivatives

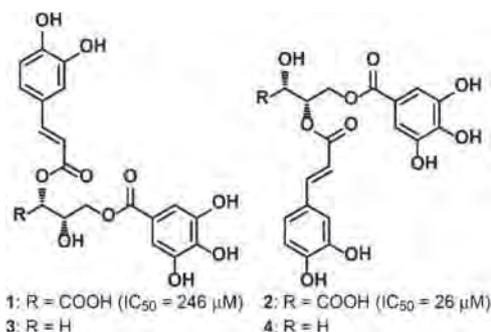
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Obesity often participates, at least partially, in the reason for metabolic disorders. Several anti-obesity drugs such as Orlistat or Centialistat have been developed, and are currently in use or in clinical trials. The target of these drugs is one of the digestive enzymes: pancreatic lipase. Lipid taken as a meal are hydrolyzed by pancreatic lipase and then absorbed from the intestine. Anti-obesity drugs target this mechanism and stop the hydrolysis of lipids to prevent their absorption.

In our effort to find a natural lipase inhibitor, screening of plants harvested in the northern area of Japan showed that the extract of *Filipendula kamtschatica* has high inhibitory activity. *F. kamtschatica* has been used by Ainu people to treat eczema and hives or as an antidiarrheal agent. Although no reports on anti-obesity effects have been reported, only a few chemical studies are reported for this plant, making it an attractive target in the search for new lipase inhibitors.

Solvent partition, chromatographic separation, and HPLC purification of this extract gave the two lipase inhibitors **1** and **2** constructed from L-threonic acid, caffeic acid, and gallic acid. The two compounds are regioisomers of caffeic acid, but the inhibitory activities of these two are reflected in IC₅₀ values of 246 and 26 μ M, with compound **2** being tenfold more active than **1**. As **1** and **2** resemble triglyceride, the substrate of lipase, we hypothesized that this inhibition is due to their structure. And for lipase, the degree of importance between three esters varies, resulting in the difference of inhibitory activity between the two isolated compounds. To investigate this and also to determine the effect of the carboxyl group, we synthesized the related compounds **3** and **4** without the carboxyl group. The inhibitory activity of these two showed only small changes caused by the difference in position of an ester (IC₅₀ not yet determined). Therefore, the carboxyl group must have some significant effect on the inhibitory activity of **2**.



P348

Discovery of a Potent Dual Antagonist of Both XIAP and cIAP Using Fragment-Based Drug Discovery

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XIAP and cIAP1 are members of the inhibitor of apoptosis (IAP) protein family. Both proteins have the ability to attenuate apoptosis via inhibition of caspases and other apoptotic factors. A defining feature of this anti-apoptotic activity is the presence of three baculoviral IAP repeat (BIR) domains in the protein sequence of both XIAP and cIAP1. The mitochondrial protein SMAC (second mitochondrial activator of caspases) deactivates the anti-apoptotic function of IAPs via a protein-protein interaction through binding of a tetrapeptide motif (AVPI) within the SMAC N-terminal region to the IAP-BIR domains.

Several companies and academic groups have active programs developing SMAC peptidomimetic compounds based on AVPI for the treatment of cancer. In general, those compounds have the tendency to be cIAP1 selective like their tetrapeptide progenitor AVPI (IC₅₀ values for XIAP-BIR3 and cIAP1-BIR3 are 0.3 and 0.016 μM, respectively).

Using our fragment-based screening approach Pyramid™, we identified a non-alanine fragment that binds with similar affinity to both XIAP and cIAP1. Hit optimisation using a structure-based approach led to the discovery of a dual XIAP and cIAP1 antagonist with optimal physicochemical properties and potent in vivo pharmacodynamic activity via the oral route. The compound achieved high concentrations in tumor and plasma over a 24 h period which ensured potent inhibition of both XIAP and cIAP1 with consequent induction of apoptosis markers (cleaved PARP, cleaved caspase-3) and strong inhibition of tumor growth.

P350

Interaction Profiling of Mephedrone at the Human Serotonin and Dopamine Transporters

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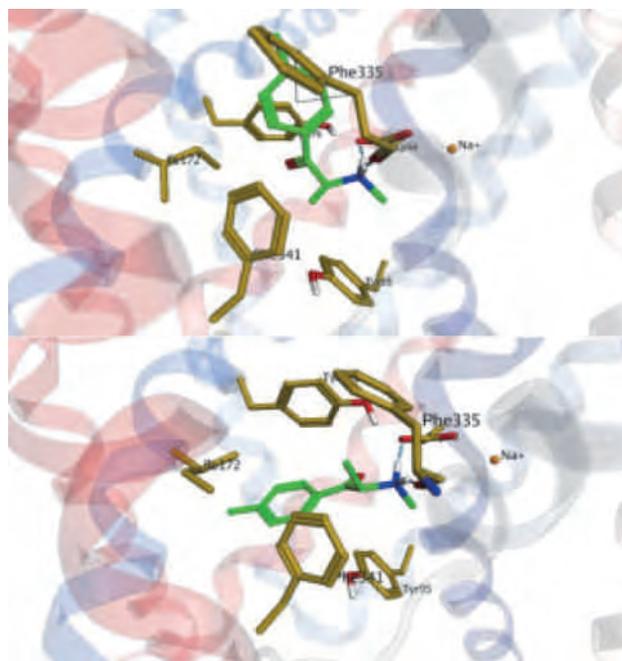
The serotonin and dopamine transporters (SERT and DAT) are transmembrane proteins that regulate the extracellular concentration of neurotransmitters by selectively re-uptaking them from the synaptic cleft. Differences in substrate selectivity patterns between these transporters lead to different behavioral phenotypes and are therefore involved in a variety of psychiatric symptoms. A better understanding of the molecular basis of substrate-transporter binding and selectivity will thus pave the way for the discovery of more selective modulators.

In this study we elucidate binding hypotheses of mephedrone, an amphetamine analogue with increasing reports of abuse, at the human SERT and DAT. First, we constructed homology models of both transporters in the occluded conformation by using the crystallized leucine transporter (LeuT) from *Aquifex aeoleus* as a template (PDB ID: 2A65). Optimal docking parameters were identified, and a variety of scoring functions were validated by performing re-docking experiments on LeuT.

Following our experimental data guided docking approach,^[1,2,3] a small library of amphetamine substrates was docked with the software GOLD, keeping the side chains in the binding site flexible. The docked poses were energy minimized, RMSD matrices were calculated, and agglomerative hierarchical clustering was performed, resulting in two possible binding modes of mephedrone (perpendicular or parallel to the membrane). The interactions were homologous in both transporter models: besides the ionic interaction of D98^{SERT}/D79^{DAT} with the substrate's cationic nitrogen, hydrogen bond and aromatic stacking interactions were found (see figure).

This supports electrophysiological experiments, which have shown that mephedrone is a substrate with comparable affinity for the human SERT and DAT.

Both binding modes found might be part of a dynamic transition between two low-energy states during substrate transport, but consensus scoring, previous studies^[4,5] and resolved templates^[6,7] indicate that the parallel orienting pose is more favorable than the perpendicular one. Further studies, using 3D-quantitative structure-activity relationships (QSAR) and mutagenesis studies, will provide a decisive answer.



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P351

Studying Structure–Activity Relationships in a Homology Model of the γ -Aminobutyric Acid Transporter 1

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Targeting the neurotransmitter transporters involved in the reuptake of γ -aminobutyric acid (GABA) is a well-established strategy for addressing diseases like epilepsy. However, specific targeting of GABA transporter (GAT) subtypes other than GAT-1, which is selectively inhibited by the anticonvulsant tiagabine, is not feasible, as attempts to develop subtype-selective tool substances were of very limited success.^[1,2] Therefore, we implemented a computational approach to elucidate the binding mode of GAT inhibitors.

To overcome the absence of X-ray structures for eukaryotic neurotransmitter transporters, a homology model based on the prokaryotic amino acid transporter LeuT from *Aquifex aeolicus* was built. Being aware that a sequence identity of just 25% between template and target sequence as well as the lack of about 40 template residues both at the N- and C-termini represent quite challenging conditions, the model was exhaustively validated and subsequently taken through 30 ns of molecular dynamics (MD) simulations.

A series of systematically modified tiagabine analogues with known activity was docked into ten representative snapshots of the transporter model, allowing for sampling the conformational space of side chain rotamers as well as for limited backbone movements. The docking studies revealed a common binding mode of the compounds, being able to explain activity differences by structural features of both involved binding partners.

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P352

Discovery of Hydroxybenzothiophene Ketones as New Dyrk1A Inhibitors for the Treatment of Down Syndrome-Related Alzheimer's Disease

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The *Dyrk1A* gene is located in the so-called critical region of the human chromosome 21, which is implicated in the development of Down syndrome. The third copy of this gene segment leads to a persistent overexpression of Dyrk1A and induces an imbalance between kinase and phosphatase activity. Dyrk1A phosphorylates tau protein and amyloid precursor protein. Hyperphosphorylation of tau is responsible for the formation of insoluble, stable aggregates of tau protein, resulting in the formation of neurofibrillary tangles. Neurofibrillary degeneration through the aggregation of tau protein and β -amyloidosis causes loss of neurons which leads to the pathological indications of Alzheimer's disease in individuals suffering from Down syndrome. Therefore, Dyrk1A is an interesting target for the development of therapeutic inhibitors. We identified a hydroxybenzothiophene ketone as a Dyrk1A inhibitor with an IC_{50} value in the sub-micromolar range. In further studies we envisage to elucidate the binding mode of this new compound class.

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P353

Design and Synthesis of Novel Tri-substituted Imidazoles as p38 Mitogen-Activated Protein Kinase Inhibitors

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The p38 mitogen-activated protein kinases (MAPKs) are a class of evolutionarily conserved protein Ser/Thr kinases. Initially discovered in 1994, p38 MAPKs have been shown to play key roles in the biosynthesis of inflammatory cytokines like IL-1 β and TNF- α .^[1] As such, early research on p38 MAPK inhibitors focused on the development of drugs for the treatment of inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, and psoriasis. In recent years, p38 MAPK inhibitors are increasingly reported for alternative indications such as chronic obstructive pulmonary disease (COPD),^[2] neural diseases,^[3] and cardiac diseases.^[4] One of the first few inhibitors of p38 MAPK is the triarylimidazole compound SB203580.^[5]

Herein we report the design, synthesis, and p38 MAPK inhibitory activities of a series of novel tri-substituted imidazoles as inhibitors of p38 MAPK. The compounds were designed with the aid of docking studies using the computational software ICM-VLS. In particular, we replaced the pyridin-4-yl group on SB203580 with a novel pyran-4-yl group and retained the key hydrogen bonding interaction with Met109 of p38 MAPK. Modification of the substituent at the imidazole C2 position resulted in compounds with similar p38 MAPK inhibition potencies as SB203580. These compounds can potentially be further developed into drugs for the indications mentioned above.

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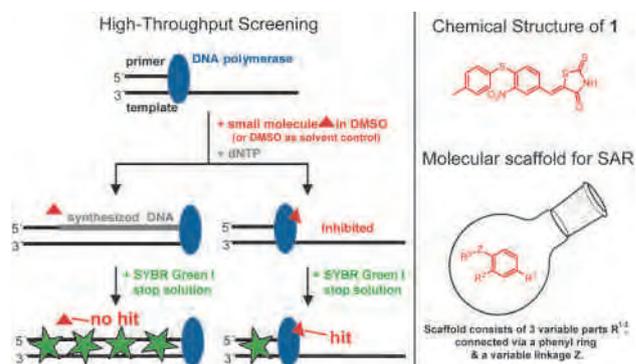
P354

Small-Molecule Inhibitors of DNA Polymerase λ and β

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The genetic integrity and survival of each organism depends on the faithful propagation of its genome. Keys for this process are DNA polymerases (pols) involved in the duplication of the genome, repair of DNA lesions, and recombination.^[1,2] In order to discover chemical probes to further understand the biological function of individual DNA pils, we established a generally applicable high-throughput screen.^[3] By applying this method we identified novel small-molecule inhibitors of the human family X DNA pils λ and β —key enzymes to maintain the genetic integrity of the genome.^[1,2] After successful confirmation of the hits, the rhodanines, classified as an excellent drug scaffold,^[4] were found to be the most potent inhibitors. Importantly, several rhodanines displayed tenfold discrimination between the two highly similar DNA pils, allowing targeted inhibition.^[3] With intent to establish basic structure–activity relationships (SAR) and to identify the core inhibitory structure of the rhodanine-based hit **1**, we subdivided **1** in a molecular scaffold and tested a number of scaffold-oriented novel analogues.^[3] Currently we initiated co-crystallization studies to get further insight into the molecular basis of inhibition of the respective DNA pils. Furthermore, the 3D structures will also facilitate structure-based optimization of the small-molecule inhibitors. Additionally the discovered small-molecule probes were investigated in a cellular context to provide insight into the function and regulation of DNA pils λ and β , and thus the phenotypic response in different human cell lines was studied. Due to the fact that DNA pils λ and β are also discussed as promising new drug targets,^[1,2] the small-molecule inhibitors reported here might serve as a starting point for the development of novel therapeutic agents.



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P355

Peptides and Pseudopeptides as SIRT6 Inhibitors

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The sirtuin gene family is highly conserved, consisting of a set of NAD⁺-dependent protein deacetylases and mono-ADP-ribosyltransferases. Sirtuins regulate several biological processes including DNA repair, apoptosis, and metabolism. Seven human sirtuins show different subcellular localization and substrate specificities, and they play crucial roles in the adaptation of cells to environmental stress. Among sirtuins, SIRT6 is localized to the nucleus, and its substrates include histone H3 and CtIP (C-terminal binding protein (CtBP) interacting protein). SIRT6 regulates glucose uptake, glycolysis, a set of genes associated with lipid storage, and the level of insulin-like growth factor 1 (IGF-1). SIRT6 has an important role in genomic stability and telomere integrity, and changes in its function can affect oncogenic transformation and tumorigenesis. In summary, SIRT6 seems to play a protective role against the metabolic consequences of diet-induced obesity, other age-related metabolic diseases, and various kinds of cancers. The potency of SIRT6 in regulating homeostasis and metabolism invites its consideration as a potential drug target.

We have previously shown that p53-based and α -tubulin-based N^F-thioacetyllysine-containing peptides and pseudopeptides are potent SIRT1 and SIRT2 inhibitors.^[1-3] In this study, we examined the potency of peptides and pseudopeptides as inhibitors of SIRT6. We tested a series of peptides and pseudopeptides in vitro and constructed a computational model of SIRT6 in order to study the binding interactions of the inhibitors. The compounds provide a basis for further SIRT6 regulator development and increase the understanding of mechanisms of SIRT6 function.

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P356

Discovery of YM178 (Mirabegron) as a Potent and Selective β 3-Adrenoceptor Agonist for the Treatment of Overactive Bladder

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The β 3-adrenoceptor (AR) has limited distribution in humans, but has an important role in the relaxation of urinary bladder detrusor tissue.^[1-3] A selective β 3-AR agonist could be a safe and effective treatment for overactive bladder (OAB) without side effects such as dry mouth, associated with antimuscarinics.^[4] In a previous report,^[5] we described a series of phenylethanolamine derivatives containing acetanilides as novel and selective β 3-AR agonists. Modification of the pyridine ring in a (2-pyridyl)acetanilide derivative as our lead compound with a heteroaromatic ring resulted in the discovery of the 2-aminothiazole moiety as a favorable pharmacophore for β 3-AR. Further optimization and evaluation with cardiovascular effects led to the discovery of YM178 (mirabegron), a (2-aminothiazol-4-yl)acetanilide derivative, as a potent and selective β 3-AR agonist, which exhibited relaxant effects in rat and human bladder strips pre-contracted with carbachol.^[6] The design, synthesis, structure–activity relationships, and pharmacological profile will be presented.

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P357

Linezolid/PA-824 Linked Co-drugs for Tuberculosis

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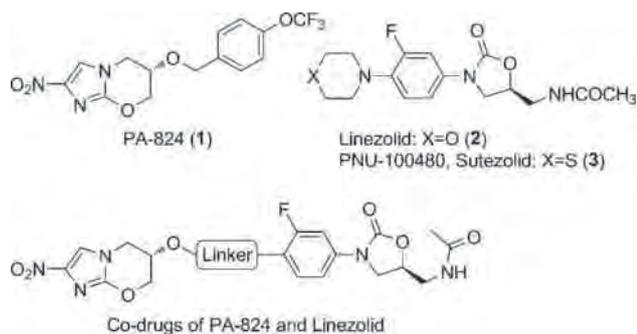
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Current treatments for tuberculosis require prolonged administration of a cocktail of drugs to achieve cure of the disease and to minimise the development of resistance. The lengths of the current protocols are due largely to the need to eradicate metabolically quiescent sub-populations of bacteria. However, such protocols are a major barrier to patient compliance, and much research is now aimed at the issues of shortening and simplifying them. New drugs are now being introduced into clinical trials that work against novel targets. For example, the (6S)-2-nitroimidazo[2,1-b][1,3]oxazines, exemplified by PA-824 (**1**) show potent activity against both replicating and non-replicating cultures of *Mycobacterium tuberculosis* (*M. tb*). Their activity against persistent *M. tb* appears due to an unusual route of reductive metabolism of the nitroimidazole ring, leading to the release of nitric oxide as a toxic and/or signalling species. This compound is currently in phase II clinical trials for the treatment of drug-susceptible and drug-resistant tuberculosis. Oxazolidinone antibacterial agents such as linezolid (**2**) and sutezolid (**3**) have been shown to have antitubercular activity, and both are in clinical trials for this indication. These drugs also have a novel mechanism of action, binding to the A-site of the 50S ribosomal subunit, thereby inhibiting early-stage protein synthesis.



In this poster we report the preparation and biological evaluation of a series of hybrid “co-drugs” of nitroimidazooxazine and oxazolidinone core units (general structure shown in figure). Some of these co-drugs had broader-spectrum antitubercular activity than either of the component compounds against resistant strains of TB, including strains separately resistant to **1** or **2** and strains doubly resistant to both. Comparative studies against equimolar mixtures of **1** and **2** also showed that some of the hybrid molecules were more active, suggestive of synergistic effects. The use of such co-drugs may also decrease the frequency of mutations leading to drug resistance. The good activity observed for these co-drugs suggests that each of the core components is still able to interact favourably with its target protein (either nitroreductase or ribosomal subunit) in these hybrid molecules.

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New Sulfonamide Derivatives as Potential Anticancer Agents

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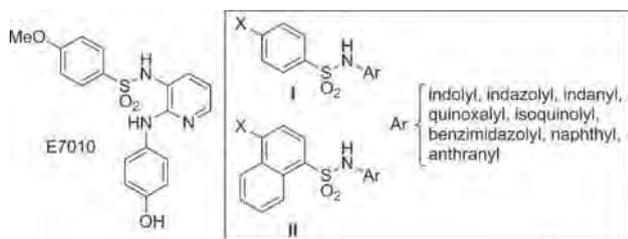
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In this communication we present a series of benzene and naphthalene sulfonamides structurally related to the sulfonamide E7010 which has entered phase II clinical trials. The synthesis and biological evaluation of benzene and naphthalenesulfonamido derivatives were carried out according to general structures **I** and **II**. The compounds were evaluated for their in vitro antiproliferative activities against a panel of eleven human cancer cell lines, including glioblastoma, colorectal cancer, non-Hodgkin lymphoma, and acute T-cell leukemia cells. Cell viability was determined by quantification of ATP, which signals the presence of metabolically active cells, using the Cell Titer-Glo Luminescent assay. The indicated human cancer cell lines were plated in 96-well plates one day before treating them with vehicle alone as a control or with the indicated compounds at 10 μM . After treatment for 48 hours, cell viability was monitored using the Cell Titer-Glo reagent. Luminescence was detected using a multi-well scanning spectrophotometer. Cell viability is represented as a percentage relative to vehicle-treated cells. Preliminary results indicate an indolyl derivative as potential anticancer lead compound. In the communication, we will report on the synthesis and biological data in detail.



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P359

Benzene and Naphthalenesulfonamides: Novel Active Compounds against *Plasmodium*, *Trypanosoma* and *Leishmania*

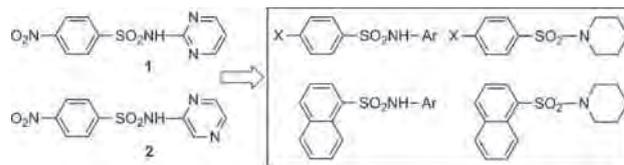
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Approximately two million people are estimated to die each year from parasitic diseases such as malaria, African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas disease) and leishmaniasis.^[1] Nevertheless, current chemotherapy has limited efficacy. Toxic side effects, route of administration, long-term treatments, and generation of resistance mechanisms highlight the urgent need to develop new drug candidates.

In a previous work^[2] we demonstrated the excellent in vivo efficacy against *L. infantum* of *para*-nitrobenzenesulfonamides **1** and **2**. In continuation of our search for candidates as antiprotozoal agents, a new series of sulfonamides has been synthesized and assayed against *Plasmodium falciparum* chloroquine-resistant strain, *T. cruzi* epimastigotes, and *Leishmania* spp. promastigotes. To establish the

selectivity index (SI), their cytotoxic effect was evaluated against the J774 macrophage cell line. The most active compounds against *Leishmania* promastigotes without cytotoxicity were tested in vitro in an amastigote model and in vivo in a murine model of visceral leishmaniasis. In this communication, we present some new naphthalene and benzenesulfonamides as potent antiprotozoal compounds.



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P361

Theoretical Study of the Interaction of β -Sultam Derivatives with DD-Peptidases

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The antibacterial activity of β -lactam antibiotics originates in their ability to inactivate DD-peptidases, a family of membrane-bound enzymes involved in the biosynthesis of the peptidoglycan essential for keeping the bacterial cell intact. In the reaction of DD-peptidases with β -lactams, a serine residue, critical for the catalytic activity of the enzyme, is acylated leading to an acyl-enzyme intermediate. This causes a decreased rate of transpeptidation, which in turn, results in a decreased synthesis of the peptidoglycan.^[1]

Research carried out by Page and co-workers^[2] has shown that some β -sultam derivatives (the sulfonyl analogues of β -lactams) can inhibit serine-dependent proteases with a mechanism of action resembling that of β -lactams. This communication presents some results on the application of computational quantum chemistry techniques^[3] to the study of the mechanism of interaction of β -sultam derivatives and DD-peptidases at the molecular level. The mechanisms of the acylation reaction of the DD-peptidases and hydrolysis of the acyl-enzyme intermediate were studied with a model of the active center of the R61 DD-peptidase,^[4] using density-

functional methods, which allow identification of the transition structures and intermediates of the potential energy surface of the corresponding reaction mechanisms. The influence on the reactivity of the structures and substituents of the various β -sultams were also analyzed.

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P362

Synthesis and Application of the First Small-Molecule Radioligand Targeting the Human Chemokine Receptor CXCR3

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The chemokine receptor CXCR3 belongs to the class of G-protein-coupled receptors (GPCRs). Chemokine receptors are activated by chemokines (chemoattractant cytokines) that regulate trafficking and effector functions of leukocytes. The endogenous ligands of the CXCR3 receptor, CXCL9, CXCL10 and CXCL11, are generally not detectable in most non-lymphoid tissues under physiological conditions, but their secretion is strongly induced by cytokines, particularly interferon- γ (IFN- γ), during infection, injury or inflammatory responses. The CXCR3 receptor is implicated in various pathological conditions, including autoimmune diseases, transplant rejection, and cancer.

The chemokine signaling system has been attracting attention of pharmaceutical companies for more than 10 years. A number of structurally diverse small-molecule negative allosteric modulators of the CXCR3 receptor have since been developed. The 8-azaquinazolinone derivatives developed by Amgen are among the best characterized. The most successful representative of the series is AMG487, which entered phase II clinical trials for the treatment of psoriasis, but failed due to the lack of efficiency.

Until now the potency of negative allosteric CXCR3 modulators was estimated by radioligand displacement assays using ¹²⁵I-labeled CXCL11 or CXCL10. We report the development and biological validation of the first radioactively labeled small-molecule allosteric modulator of the CXCR3 receptor derived from the 8-azaquinazolinone chemotype. After detailed characterization of the new radioligand RAMX3, a series of new derivatives of 8-azaquinazolinone chemotype

and reference compounds AMG487 and NBI-74330 were synthesized and characterized in the radioligand displacement assays using a novel radioligand. Additionally, all derivatives were characterized in the functional assay using the reporter gene assay and CXCL11 as the endogenous ligand.

The data obtained indicate that the correlation between affinity and biological efficacy of CXCR3 receptor ligands is not always straightforward. The novel radiolabeled allosteric modulator RAMX3 proved to be a versatile and robust tool for the discovery and investigation of allosteric CXCR3 receptor modulators. This radioligand will enable us to investigate subtle details in the binding mode of CXCR3 receptor's allosteric modulators of different chemotypes and the molecular mechanism of their action, which would not be possible with the use of traditional radiolabeled chemokines.

P363

Design and Synthesis of C-Glycosidic LpxC Inhibitors

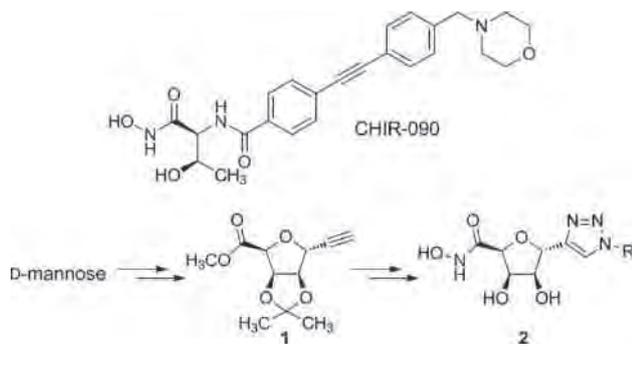
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Infections by Gram-negative bacteria remain an important concern to public health all over the world. Especially with the emergence of multidrug resistant Gram-negative bacteria, there is an urgent need for the identification of antibiotics with novel mechanisms of action to complement the existing drugs that inhibit protein, nucleic acid, or cell wall biosynthesis.

The biosynthesis of lipid A, the hydrophobic membrane anchor of lipopolysaccharide (LPS), was identified as an attractive target for the development of novel antibiotics, as lipid A is essential for the growth and viability of the majority of Gram-negative bacteria. The irreversible deacetylation of UDP-3-O-[(R)-3-hydroxymyristoyl]-N-acetylglucosamine, which is the first committed step in the biosynthesis of lipid A, is catalyzed by LpxC, a Zn²⁺-dependent enzyme. As the inhibition of LpxC is lethal to various Gram-negative bacteria, inhibitors of this enzyme can be developed as new antibiotics.

CHIR-090 is the best LpxC inhibitor reported to date, killing both *E. coli* and *Pseudomonas aeruginosa* in bacterial disk diffusion assays with an efficacy similar to that of ciprofloxacin. Therefore, CHIR-090 was chosen as lead compound for the design of conformationally constrained analogues **2**. In a chiral-pool synthesis starting from D-mannose, key intermediate **1** was synthesized in a stereocontrolled manner. The ethynyl group of this C-glycoside allows the attachment of various side chains via azide-alkyne cycloadditions, giving access to various CHIR-090 analogues **2**.



P364

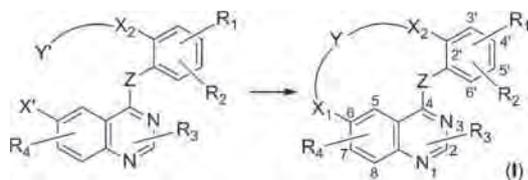
Macrocyclization of Kinase Inhibitors: Successfully Rounding Corners in Open Space

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One of the recurring problems in finding novel ATP-site-directed kinase inhibitors is the identification of patentable kinase scaffolds. Readily accessible scaffolds such as anilino-pyrimidines, quinazolines, and (aza)indoles, indazoles, and indazolones have been patented heavily, and offer little opportunity for finding new IP space. Macrocyclization^[1] of kinase inhibitors is an innovative method to create new IP space outside the existing patents describing these compounds. This approach also generates extra opportunities to explore activity and selectivity, and to modulate physicochemical and ADME properties of the 'open form' compounds.^[2] However, macrocyclization introduces structural complexity, making parallel synthesis approaches less obvious, and creates additional challenges in interpreting structure–activity and property relationships.

In this poster, we describe some aspects that contributed to the development of a novel proprietary library of macrocyclic kinase inhibitors. We discuss the design of synthetic schemes suitable for parallel synthesis, and the various possibilities applied toward the final ring-closing step. In addition, physicochemical and biological data for macrocyclic kinase inhibitors is presented in comparison with the 'open form' analogues.



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P365

Synthesis, Antitumor Activity and QSAR Analysis of Heterocyclic Amides and Quinolones

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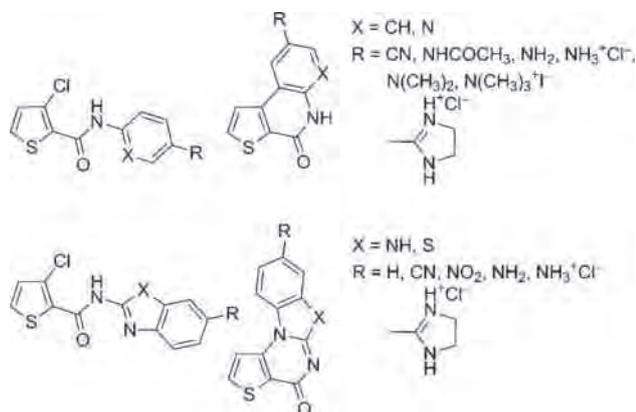
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The development of effective anti-neoplastic drugs has become one of the most intensively studied aspects of contemporary medicinal chemistry, and therefore has seen tremendous growth in the number and types of new anticancer agents. Substituted heterocyclic amides and their fused analogues, quinolones, have shown a wide range of biological activity and are still intensively investigated. We previously reported the synthesis and strong inhibitory activities toward several human cell lines of various amidino-substituted benzo[*b*]thiophene-2-carboxamides and benzo[*b*]thieno[2,3-*c*]quinolones.

In this report we present the synthesis, antiproliferative activity, and QSAR analysis of some novel thiophene-2-carboxamides and their fused quinolone analogues as part of our continuing research into polyfunctional heterocyclic compounds. For the synthesis of novel compounds, we used both classical methods of organic synthesis as well as photochemical procedures. Prepared compounds were tested for their antitumor activity toward several human tumor cell lines. Using measurements of antitumor activity of heterocyclic amides and quinolones 3D-QSAR models were built. A Volsurf-based procedure, which is independent of the alignment of molecules, was used. Using this knowledge, new compounds were proposed for synthesis and biological testing. Their activities were predicted with the derived QSAR models, and the proposed compounds were shown as promising antitumor candidates.



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P366

Antiproliferative Evaluation of Some New Amidino-Substituted Bisbenzothiazolopyridines

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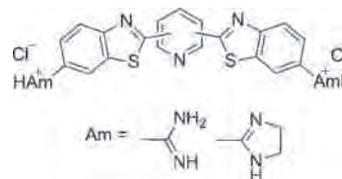
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Classical chemotherapy, based on use of small molecules or bioactive natural products, is still the mainstay for cancer treatment that aims at major cellular targets such as DNA, tubulin, or protein kinases.^[1,2] Even though chemotherapy remains the standard cancer therapy option, the use of available chemotherapeutics is rather limited due to severe side effects or a limited choice of available anticancer drugs. This clearly underscores the need for the development of more effective cancer treatments and new classes of chemotherapeutics. Benzothiazole derivatives are of considerable interest due to diverse pharmaceutical properties; for example, substituted 2-arylbenzothiazoles have recently emerged as a privileged scaffold in drug discovery, as they bear remarkable activity profiles in noninvasive diagnosis of Alzheimer's disease and antitumor effects.^[3] As a continuation of our recent studies,^[4] we present the synthesis and biological evaluation of newly synthesized amidino-substituted conformationally restricted derivatives of bis-benzothiazolopyridines (shown).

Biological evaluation of obtained compounds revealed different antiproliferative potential among diamidino and diimidazolonyl derivatives, the latter exerting stronger concentration-dependent antiproliferative effects on the tested panel of tumor cell lines. The presence of an imidazo moiety increased the selectivity for some tumor cell lines, while both imidazo and imidamide moieties contributed to increased antiproliferative effects on the growth of MCF-7 and MiaPaCa-2 cell lines. Chemoinformatics analysis revealed possible severe hERG channel blockade as a side effect of the tested compounds. Therefore, their potential lies in local administration directed toward chosen biological targets.



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P367

'Traceless' Prodrugs for Peptidomimetic Caspase Inhibitors

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The use of prodrugs as a method for improving the ADME properties of a potential drug candidate are well documented. However, in some cases the liberation of a stoichiometric amount of the pro-moiety can lead to toxicity concerns. To overcome this potential pitfall, a series of prodrugs based on a 6,6a-dihydrofuro[3,2-d]oxazol-5(3aH)-one motif were designed as 'traceless' prodrugs for caspase inhibitors. In vivo hydrolysis of the lactone functional group is sufficient to liberate the active inhibitor in vivo and in vitro without producing any cleavage by-product. When dosed orally in rats, an improvement in oral availability is observed. These results suggest such prodrugs may have the potential to be used to enhance the systemic exposure of peptidomimetic caspase inhibitors in indications where oral administration is desirable.

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Synthesis and Biological Evaluation as Microtubule-Active Agents of Several Lactone and Spiroketal Derivatives

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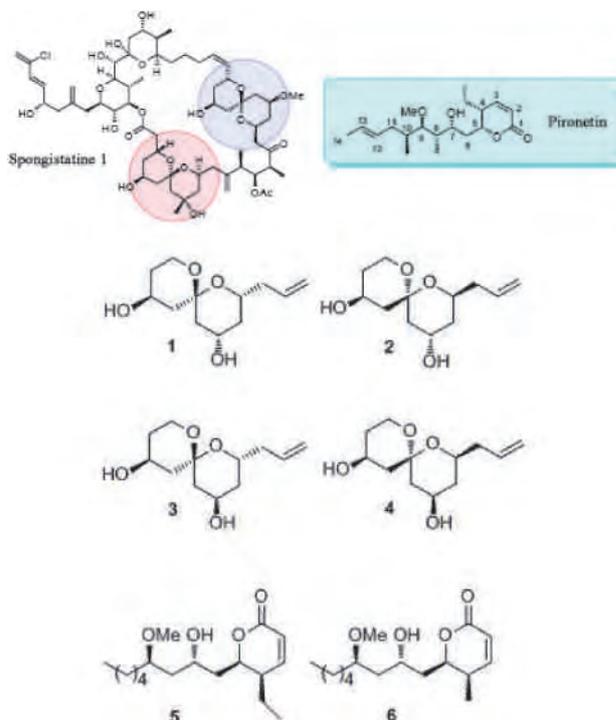
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Microtubules are dynamic polymers that play a central role in cell division, as they are key constituents of the mitotic spindle. They are composed of protein heterodimers formed by two similar polypeptides: α - and β -tubulin. As tubulin-binding molecules (TBMs) such as spongistatine 1 and pironetin constitute a very important class of anticancer agents,^[1] we have become interested in investigating the synthesis and biological properties of nonnatural TBMs with comparatively simple structures and the ability to selectively bind α - or β -tubulin.

Toward the synthesis of β -tubulin-binding molecules, we designed several derivatives taking spongistatin 1 as the reference model. This natural compound, which is a very potent inhibitor of the growth of many cancer cell types,^[2] contain two 6,6-spiroketal fragments, which could form close contacts with certain protein residues in the pocket. This led us to undertake the stereoselective preparation of several structurally simple molecules containing the aforementioned structural fragments of the spiroketal type (**1–4**). On the other hand, pironetin, an α -tubulin-binding molecule, proved a potent inhibitor of tubulin assembly and was found to arrest cell cycle progression in the G₂/M phase.^[3] This feature has motivated us to undertake the synthesis of simplified analogues (**5–6**) of this natural compound, in which two alkyl groups and a double bond were removed.

To check the cytotoxicity of our compounds, we determined the corresponding IC₅₀ values on A2780 and A2780AD (MDR-overexpressing P-glycoprotein) human ovary carcinomas. We also studied the effect of them on the cytoplasmic microtubule network and on DNA of A549 lung carcinoma cells, as well as the critical concentration required for tubulin assembly determined in GAB.



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P369

Angiogenesis Inhibitor for the Treatment of AMD by Topical Application

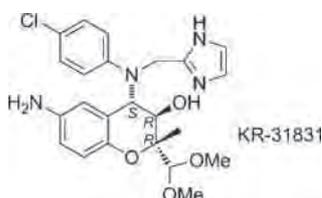
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Age-related macular degeneration (AMD) is the leading cause of vision loss in people over the age of 60. The United Nations estimates that approximately 20–25 million people worldwide suffer from AMD. Because the formation and growth of new blood vessels play a major role in the disease pathogenesis of wet AMD, treatment with anti-angiogenic therapies has strong scientific and clinical rationale. The administration of VEGF inhibitors into the vitreous is very effective in treatment of ocular angiogenesis. The VEGF antibody ranibizumab (Lucentis) was approved by the FDA for CNV-AMD in 2005.

KR-31831, a new synthetic small-molecule benzopyran derivative,^[1] exhibits strong anti-angiogenic action and has the potential to be a useful inhibitor of the large number of serious diseases characterized by upregulated angiogenesis. KR-31831 suppresses the angiogenic action of endothelial cells as determined by in vitro

and in vivo angiogenesis assays.^[2] KR-31831 inhibited the proliferation, migration, invasion, and tube formation in vitro and inhibited in vivo angiogenesis in mouse Matrigel plug assays.^[3] Furthermore, KR-31831 decreased the mRNA expression of bFGF, FGFR-2, and VEGFR-2. KR-31831 showed excellent inhibitory effects of corneal neovascularization similar to Avastin, a monoclonal antibody. The compound exhibited good efficacy in a laser-induced choroidal neovascularization model at two weeks after intraperitoneal injection and inhibited lymphangiogenesis in silver nitrate cauterized cornea. The topically delivered compound as eye drops exhibited good ocular pharmacokinetics, and reaches the back of the eye in sufficient concentrations for the treatment of back-of-the-eye diseases, as in the case of AMD. No small-molecule VEGF antagonist for AMD is in the market at present, so KR-31831, a small-molecule anti-angiogenic agent, has the potential to be useful in therapeutics for AMD.



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P370

Inhibition of *Leishmania infantum* Trypanothione Reductase by Designed Peptides Targeting the Dimerization Interface

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Leishmaniasis is a parasitic infection caused by unicellular protozoan organisms (*Leishmania* spp.) belonging to the Trypanosomatidae family. Although leishmaniasis is emerging nowadays as a world-wide disease,^[1] currently available drugs are decades old, and their efficacy and safety profiles leave much to be desired.^[2] There is an urgent need to find new targets and/or inhibition mechanisms against these parasites.

Trypanothione reductase (TryR) is an essential enzyme for survival in trypanosomatids that is used to maintain the dithiol of trypanothione [bis(glutathionyl spermidine)] in its reduced state. Because of the lack of catalase and glutathione peroxidase activities, these parasites rely solely on the trypanothione system as a defense mechanism against the oxidative stress that is generated either by their own metabolism or by the host immune response. This fact makes TryR a very attractive target for drug development against trypanosomatid-caused infections.^[3]

Most, if not all, the efforts made by the scientific community to inhibit TryR have relied on the design of molecules directed toward the active site, but they have not been very successful. For this reason, and based on the fact that the functional form of TryR is a homodimer, we have devised a yet unexplored alternative strategy that attempts to disrupt the dimer interface. The most relevant residues in the dimerization process of the enzyme were first identified by computational analysis of the three-dimensional structure of *Leishmania infantum* (Li-TryR).^[4] The selected residues were next validated as hotspots by site-directed mutagenesis studies.^[5]

On the basis of these results, we herein describe the design, synthesis, conformational studies and biological evaluation of a small library of linear peptides that mimic the interfacial α -helix defined by residues Pro435–Met447 as putative dimerization inhibitors of Li-TryR. With the aim of increasing α -helical propensity, a second series of hydrocarbon-stapled^[6] analogues of the most active linear peptides was also prepared and studied. The ability of the peptides to interfere with TryR dimerization and activity was assessed in vitro, and NMR conformational studies and circular dichroism measurements of selected peptides were carried out. Our results support that targeting the protein–protein interface of Li-TryR by rationally designed interfacial α -helical peptides is a feasible goal.

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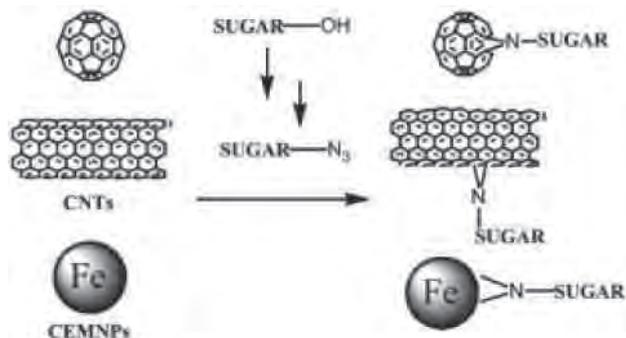
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Approach to Functionalization of Carbon Nanoparticles with Sugar Azides

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The chemical functionalization of fullerenes, carbon nanotubes (CNTs), and carbon-encapsulated magnetic nanoparticles (CEMNPs) is a prerequisite for the use of these synthetic carbon allotropes in high-performance applications, for example in medicine^[1] or materials science.^[2] In our studies we are trying to develop a wide amount of species based on carbon allotropes with unique properties. Modifications of carbon spheres are carried out with sugar azides in pericyclic addition. Modified carbon nanoparticles are characterized by increased solubility and can be used as a new class of MRI contrast agents, antioxidants, etc.



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Molecular Modelling and Simulation Studies of MurA Enzyme: towards Novel Mur Enzyme Inhibitors against Resistant Bacterial Strains

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Introduction: As infectious diseases are one of the world's leading causes of death, increasing antibacterial resistance poses a global threat for human health. Thus, there is an urgent need for novel antibiotics that are effective against pathogens such as multiresistant *Staphylococcus aureus*.^[1] One conventional goal of antibiotics has been to inhibit the biosynthesis of peptidoglycan, the main element of the bacterial cell wall.^[2] Most of the current antibiotics that disrupt the bacterial cell wall interfere with the extracellular steps of peptidoglycan biosynthesis, but the intracellular synthesis steps leading to the assembly of the peptidoglycan monomer are still poorly exploited. The MurA–F enzymes are attractive target candidates for drug development, as they catalyze the intracellular synthesis steps of peptidoglycan.

The present work focuses on MurA, the first enzyme in the 'Mur assembly line'. It transfers the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to UDP-*N*-acetylglucosamine (UNAG). Binding of the MurA substrates induces a large conformational change where a flexible loop (residues 112–121) closes the active site by covering it as a lid. Ligands other than the substrates can significantly affect the conformation of the loop.^[3] There is only one clinically used MurA inhibitor to date: fosfomycin. Unfortunately, development of resistance to fosfomycin is not rare and, therefore, novel inhibitors of MurA are needed. Moreover, targeting two or more Mur enzymes at the same time should decrease the risk of developing antibiotic resistance. Herein we present a multi-pronged modeling study of MurA to provide insight into designing novel more effective antibacterials.

Methods: The following steps were taken to gain an understanding of the function of MurA and to discover novel Mur enzyme inhibitors. 1) The ligand-protein interactions of the available MurA crystal structures from the Protein Data Bank were analyzed using PyMOL. 2) The conservation level of all the residues interacting with ligands was studied with the ConSurf server to gain a comprehensive overview of the possible important target sites for the novel MurA inhibitors. The more conserved the residue, the less likely it is to be a resistance-causing mutation. 3) The enzyme dynamics related to ligand binding was studied by normal mode analysis and standard/enhanced molecular dynamics (MD) simulations of the apo enzyme as well as particular ligand–MurA complexes. 4) Virtual screening of novel MurA inhibitors was performed by docking the National Cancer Institute diversity set III into the MurA substrate binding pocket and a previously reported allosteric site

with the GOLD docking program. The same compounds were also docked at the MurB substrate binding site to find any common inhibitor candidates that could act as dual MurA+B inhibitors. 5) UNITY and Surflex-Sim database screening tools were applied to search for compounds that have the pharmacophoric features of a reported allosteric inhibitor. Subsequently, the UNITY/Surflex-Sim hits were also docked to the MurA+B structures. In the analysis of the docked ligands we focused on the compounds that are ranked high in both enzymes to choose the most promising virtual hits for experimental testing.

Results: The present study sheds light on MurA as an important antibacterial target and its use in drug design. On the basis of the modeling and simulation results, we will discuss the possible alternative binding sites for novel inhibitors. The virtual hit compounds discovered through this work will be ordered and experimentally tested for their inhibitory effect on MurA and MurB.

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P373

Structure–Activity Relationships of Novel BK Channel Modulators: The GoSlo-SR Family

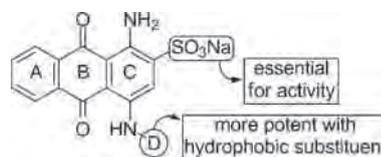
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Large-conductance potassium ion channels (BK channels), also called Maxi-K, are membrane-associated ion channels, which conduct K⁺ ions across a cell membrane. BK channels are present in a wide variety of tissues throughout the body and are activated (opened) or deactivated (closed) by two physiologically relevant factors; a change in intracellular Ca²⁺ ion concentration or a change in the electrical potential across the cell membrane. Drugs that activate or open BK channels are in high demand for their potential clinical use. Primary indications for BK channel openers include urinary incontinence, arterial hypertension, erectile dysfunction and airway constriction.

We have designed and synthesized novel anthraquinone analogues called the GoSlo-SR family, that open BK channels when applied to the internal surface of excised patches of membrane from rabbit urinary bladder smooth muscle cells (UBSMC). Excised patches were held at –60 mV, and BK channel currents were evoked using voltage ramps (100 mV sec⁻¹). Single channel currents from UBSMC had a large conductance (335±5 pS, mean±SEM, n=9), reversed at the K⁺ equilibrium potential (0 mV) and were abolished in the presence of the BK channel blocker penitrem A (100 nM, n=5). Compounds were synthesized under microwave irradiation using the copper-catalyzed Ullmann coupling reaction with the commercially available bromoaminic acid and the appropriate amine.^[1] The effects of all compounds were screened at a concentration of 10 μM.

We first altered the number of carbons in the aliphatic D ring and examined the effect of each compound on ΔV_{1/2} in the presence of 100 nM Ca²⁺. Increasing the ring size from cyclopropane to cycloheptane, shifted ΔV_{1/2} by +8±6 mV (n=5), –17±6 mV (n=5), –24±6 mV (n=4), –54±8 mV (n=4), and –61±6 mV (n=6), respectively. Substitutions of the aliphatic D ring (ΔV_{1/2} ~ –54±8 mV, n=4) with a six-membered aromatic ring (ΔV_{1/2} ~ –51±10 mV, n=4) did not alter the efficacy. However, in all subsequent syntheses, the suitable aniline derivatives were used to produce a library of compounds to study the structure–activity relationship (SAR) of the novel BK channel modulators.^[2]



We examined the effects of different substituents on the D ring and found that the addition of electron-withdrawing groups (NO₂, F) or hydrophilic groups (CN, SO₃ H, CO₂ H) at the *meta* position significantly decreased efficacy and thus shifted ΔV_{1/2} by only 1±23 mV (n=3), –28±13 mV (n=5), –13±6 mV (n=5), –16±9 mV (n=4), and –23±13 mV (n=3), respectively. In contrast, hydrophobic substituents at the same position, such as ethyl, isopropyl, and *tert*-butyl increased efficacy and shifted ΔV_{1/2} by –92±11 mV (n=9), –120±15 mV (n=7), and –113±5 mV (n=6), respectively. Enhancing the lipophilicity of the phenyl ring with *meta*-CF₃ and *para*-methyl groups produced the most efficacious member of this family, GoSlo-SR-5-44, which shifted the ΔV_{1/2} by –142±8 mV (n=12), an effect that was significantly greater than that observed with the *meta*-CF₃ substituent alone, GoSlo-SR-5-6 (–107±7 mV, n=12, p<0.05). Concentration effect curves were obtained to compare the potencies of these two compounds and the EC₅₀ was 2.4 μM and 2.3 μM for GoSlo-SR-5-44 and GoSlo-SR-5-6, respectively. The effects of GoSlo-SR-5-6 were abolished in the presence of penitrem A (100 nM, n=6). These results demonstrate that the GoSlo-SR family of compounds is a novel class of BK channel openers and that increasing the hydrophobicity of the D ring enhances their efficacy.

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Synthesis, Antitumor Activity In Vitro and Interaction with DNA of Novel Amino-Substituted Benzimidazo[1,2-*a*]quinolines

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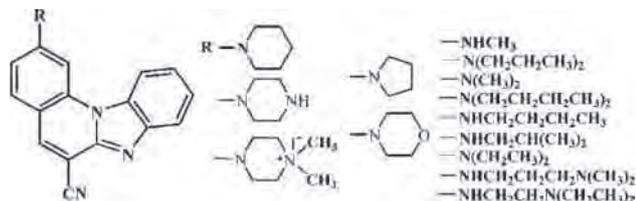
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Over the past few years substituted benzimidazoles and their azino-fused derivatives have been one of the most extensively studied classes of heterocyclic compounds due to their well-known biological activities. Benzimidazole nuclei have been widely incorporated in the structures of numerous important medical and biochemical agents. Because of the structural similarity with some naturally occurring compounds such as purines, benzimidazole derivatives can easily interact with biomolecules in living systems. Azino-fused derivatives, due to their planar structure and interesting fluorescence properties, have the ability to intercalate into DNA/RNA molecules and could offer a potential application as fluorescent probes in homogeneous assays of biological systems for detection of biomolecules in biomedical diagnostics.

As a part of our continuing research in the field of medicinal chemistry, novel amino-substituted benzimidazo[1,2-*a*]quinolines were synthesized. For the preparation of targeted compounds, besides classical reactions of organic chemistry, photochemical and microwave-assisted synthesis were used. All compounds were characterized by ¹H and ¹³C NMR, IR, UV/Vis and fluorimetric spectroscopy. Antitumor activity in vitro of prepared compounds was tested on breast, colon, and lung carcinoma cell lines. All compounds showed mostly prominent antiproliferative effect on the tested panel cell lines with GI₅₀ concentrations in the sub-micromolar range. To shed more light on the mechanisms of biological action, additional experiments of interaction with DNA of the most active compounds were performed.



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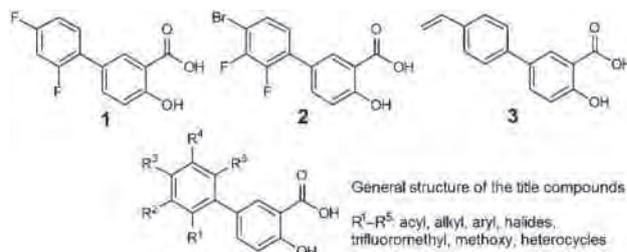
Analogues of Diflunisal as Inhibitors of Bacterial Hyaluronidases

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Hyaluronidases are enzymes that degrade hyaluronan, a major constituent of the extracellular matrix. As the role of hyaluronidases is far from being understood, potent and selective inhibitors are required as pharmacological tools. Moreover, inhibitors of the bacterial and mammalian enzymes might be useful agents in the treatment of various diseases such as bacterial infections.^[1] Previously, we identified hyaluronidase inhibitors among vitamin C derivatives, indoles and benzoxazoles.^[2] Characteristics of the most active inhibitors are acidic functional groups and a high degree of lipophilicity, resulting in very high plasma protein binding.

Aiming at more drug-like molecules, we investigated several commercially available nonsteroidal anti-inflammatory drugs (NSAIDs) purported to have hyaluronidase-inhibitory activities,^[3] for inhibition of bacterial hyaluronidase from *S. agalactiae* (*Sag* Hyal₄₇₅₅). We identified diflunisal **1**, with an IC₅₀ value in the micromolar range (IC₅₀ 194 μM) as a screening hit. The inhibitory activity of diflunisal was also determined in the presence of human plasma and fetal calf serum (FCS) in order to investigate inactivation due to protein binding. No differences in IC₅₀ values relative to the assay conditions in the absence of human plasma or FCS were detected. Therefore, we synthesized a series of salicylic acid derivatives, which are structurally related to **1**, with different substitution patterns (see general structure). The title compounds were synthesized via Suzuki coupling.^[4]



In a turbidimetric assay for inhibition of *Sag* Hyal₄₇₅₅, IC₅₀ values in the two-digit micromolar range were determined at the pH optimum (pH 5) of *Sag* Hyal₄₇₅₅, e.g., for 4'-bromo-2,3'-difluoro-4-hydroxybiphenyl-3-carboxylic acid (**2**) (IC₅₀: 28 μM) and 4-hydroxy-4'-vinylbiphenyl-3-carboxylic acid (**3**) (IC₅₀: 23 μM). In summary, these compounds may be considered lead structures in the search for hyaluronate lyase inhibitors as pharmacological tools and potential drugs.

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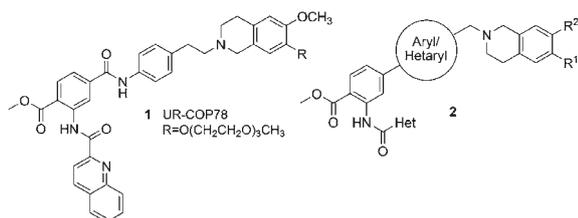
N-Acylated Anthranilic Acid Esters: Highly Potent and Selective Modulators of Breast Cancer Resistance Protein (BCRP, ABCG2)

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ABCG2 (BCRP), an ATP-driven drug efflux transporter, is, among other ABC proteins, responsible for multidrug resistance (MDR) in cancer.^[1] Furthermore, as a fundamental constituent of the blood–brain barrier, ABCG2 limits the efficacy of different potent cytostatics (e.g. topotecan) in the treatment of malignant tumors of the CNS. Consequently, by analogy with an approach described for ABCB1,^[2] co-administration of ABCG2 modulators and appropriate cytostatics is an attractive strategy to treat MDR tumors and to improve the chemotherapy of malignancies in the CNS by increasing the drug levels in the brain.^[3,4]

Recently, we synthesized a series of tariquidar analogues, which were surprisingly identified as potent and selective ABCG2 modulators.^[5] Increased water solubility and higher maximum ABCG2 inhibition were achieved with compounds such as UR-COP78 (**1**) comprising triethylene glycol groups.^[6] Whereas the ester group of these compounds turned out to be stable in mouse plasma, unfortunately, the benzamide group was enzymatically degraded within 30 minutes. Aiming at higher stability in view of future in vivo studies, the *N*-phenylcarboxylic amide moiety was replaced by putative bioisosteres, resulting in new classes of modulators containing a biaryl or a heterocyclic core, respectively (**2**).



The synthesized modulators were investigated for inhibition of ABCB1 and ABCG2 in a calcein-AM and a Hoechst 33342 microplate assay using ABCB1-overexpressing Kb-V1 and ABCG2-overexpressing

MCF-7/Topo cells. All compounds showed a very high maximal inhibitory effect of 80–110% relative to fumitremorgin C (10 μM) in the Hoechst 33342 assay. Bioisosteric replacement of the the benzanilide moiety by heterocycles turned out to be most successful. At present, with respective IC_{50} values of 60 and 46 nM and maximal inhibitory effects of 100 and 110%, UR-COP269 and UR-COP272 are the most promising compounds. At the ABCB1 transporter both compounds showed only marginal activity.

Representative new modulators were investigated for stability in mouse plasma. For each compound only one cleavage product was identified: the free carboxylic acid resulting from ester hydrolysis. In all cases, degradation became obvious after 30–60 minutes; after 5 hours ~80–90% of the parent compound was still detected, and between 50 and 70% of the ester was still present after 24 hours.

Among the title compounds, highly potent ABCG2 modulators were identified, which may have the potential to increase drug levels in the CNS. The improved stability, increased solubility, and ABCG2 inhibitory efficacy of these new modulators relative to the previously described compounds^[5] are very promising with respect to in vivo studies using refined orthotopic xenograft models in nude mice.

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P377

Noraristeromycin Inhibited Cell Proliferation and Motility, and Led to Apoptosis of Human Prostate Cancer Cell Lines

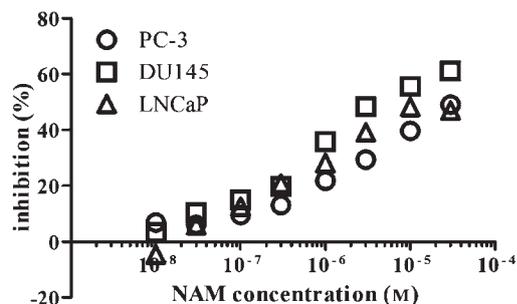
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IKK kinase α (IKK α) has been identified as a key mediator of inflammation and metastasis in prostate cancer. However, it was not clear whether IKK α inhibition suppresses the growth of prostate cancer cells because there had been no potent and selective small-molecule IKK α inhibitor. We previously reported that noraristeromycin (NAM) effectively regulates the NF- κB activation pathway by specifically inhibiting IKK α in vitro.^[1]



In this study, we investigated the effect of NAM on cell proliferation, motility and apoptosis in both androgen-independent (PC-3 and DU145) and androgen-dependent (LNCaP) human prostate cancer cell lines. Treatment with NAM suppressed the cell proliferation of PC-3, DU145, and LNCaP in a dose-dependent manner. In addition, NAM inhibited cell motility and led to apoptosis at concentrations >3 μM. These results suggest that IKKα inhibition is an effective novel therapeutic goal for the treatment of prostate cancer.

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P378

Diversity Oriented Synthesis of New Cyclopeptide HDAC Inhibitors for Fighting Apicomplexa Parasites

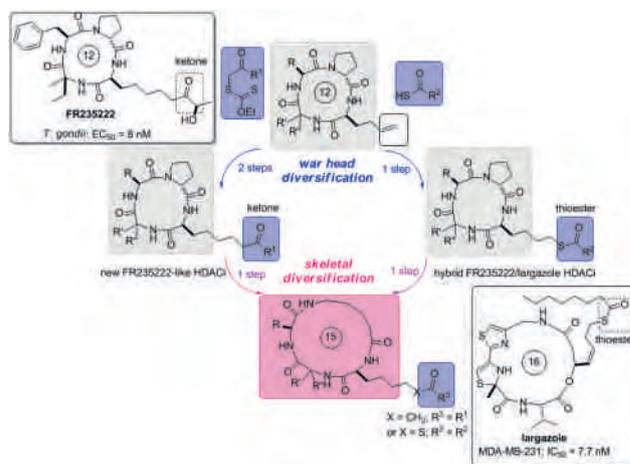
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Recently we identified the natural product cyclopeptide FR235222 as a highly potent histone deacetylase inhibitor (HDACi), efficient at 8 nM on *Toxoplasma gondii*, the apicomplexa parasite responsible for toxoplasmosis, albeit with a poor selectivity index (SI=12).^[1] We demonstrated that the inhibition of histone deacetylase HDAC3 with this HDACi induced epigenetic modifications, which strongly affected strain virulence at the tachyzoite and bradyzoite stages of the parasitic life cycle.^[1,2] This dual efficiency with a bioactive molecule on two distinct stages of the parasitic life cycle is unprecedented and outlines the key potential of targeting the epigenetic mechanisms to control parasite proliferation. The design of easily adjustable/customizable syntheses and according to the principles of diversity oriented synthesis (variations on appendages and skeletal diversities) is essential to decrease the time in the

optimization process by direct access to structurally diverse and relevant bioactive products. In this communication we present our synthetic strategy involving post-transformation reactions on a common cyclopeptide scaffold that affords new bioactive HDACi in only one or two steps. These new structures combine structural features ranging from FR235222 to largazole and have shed light on important structure–activity relationships. As a result, we managed to optimize new synthetic cyclopeptide HDACi that retain their efficacy against the parasite at 10–20 nM and with better selectivity indexes (SI=40–60).



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P380

Studies Toward the Interaction of HRV 3C Protease with (–)-Thysanone

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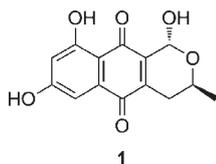
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More than 500 million cases of common colds occur each year in America alone, costing society an estimated US\$40 billion.^[1] The major cause for this common disease is the human rhinovirus (HRV), and currently only symptomatic treatment is available to medicate these infections.^[2] The RNA genome of HRV encodes 12 different

structural and non-structural proteins/enzymes which are expressed intracellularly as one polyprotein that undergoes subsequent proteolytic processing.^[3,4] In HRV, these cleavages are mainly governed by two virally encoded cysteine proteases designated 2A and 3C.^[3,4] The action of HRV 3C protease is required to produce mature viral proteins and functional viral enzymes essential for completion of viral replication.^[2-9]

(-)-Thysanone **1** exhibits potent inhibition of HRV 3C protease (IC_{50} 13 $\mu\text{g mL}^{-1}$),^[10] and it is a promising lead for the development of novel chemotherapeutic agents for control of the common cold. In this current study, we synthesised (-)-thysanone **1** and a series of analogues with varied stereochemistry, heteroatom position, and substitution pattern. Furthermore, we developed a novel fluorescence-based assay to test compounds quantitatively for activity against HRV 3C protease for determination of structure–activity relationships. Mechanistic studies toward the mode of interaction of HRV 3C protease with (-)-thysanone **1** and its analogues were also performed. This information will be used to design improved inhibitors for HRV 3C protease for treatment of the common cold.



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P381

Synthesis, In Vitro, In Vivo, and Computational Studies of Azaheterocycles with Anti-hyperglycemic Activity

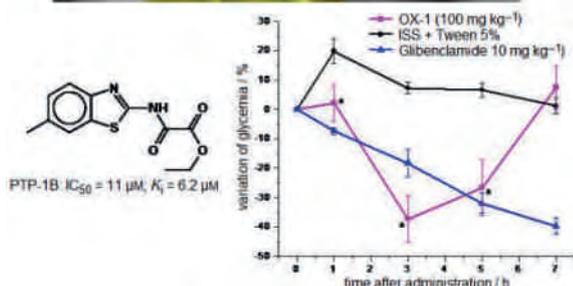
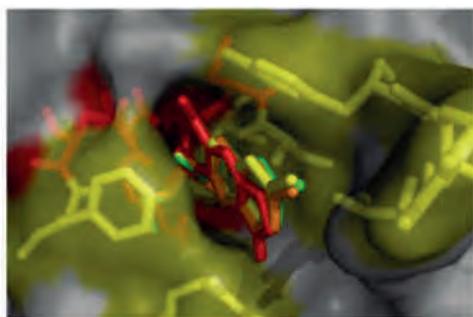
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The ethyl 2-(6-substituted benzo[d]thiazol-2-ylamino)-2-oxoacetate derivatives (**OX-1–9**) were prepared using a one-step reaction. The in vitro inhibitory activity of the compounds against protein tyrosine phosphatase 1B (PTP-1B) was evaluated. Compounds **OX-1**, **OX-6**, and **OX-7** were rapid reversible (mixed-type) inhibitors of PTP-1B, with IC_{50} values in the low micromolar range. The most active compounds **OX-1**, **OX-6**, and **OX-7** were docked into the crystal structure of PTP-1B. Docking results indicate potential hydrogen bond interactions between the oxamate group in all compounds and the catalytic amino acid residues Arg221 and Ser216. The compounds were evaluated for their in vivo hypoglycemic activity, showing significant lowering of plasma glucose concentration in acute normoglycemic model and oral glucose tolerance test similarly at the effect exerted for hypoglycemic drug glibenclamide.

On the other hand, 2-{2-[(naphthalen-1-ylsulfonyl)amino]-1,3-thiazol-4-yl}acetamide derivatives were prepared using a short synthetic route. The in vitro inhibitory activity of the compounds against 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) was evaluated. Compounds **GMV-5** and **GMV-10** had moderate inhibitory activity (55.26 and 67.03% inhibition at 10 μM , respectively) and were more active than **BVT-14225** (positive control). Both compounds bear a piperidine ring in their structure, and were selected to establish their in vivo antidiabetic effect. The in vivo evaluation was performed using a type 2 diabetes mellitus rat model determined at 100 mg kg^{-1} single dose. The results indicated a significant decrease of plasma glucose levels. Additionally, we performed a molecular docking of compounds into the ligand binding pocket of one subunit of human 11 β -HSD1. In this model binding, the oxygen atom of sulfonamide forms a hydrogen bond with the catalytic residues Ser170, Ala172, and we observed important π - π interactions between the naphthyl group and Tyr177.^[1]



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P382

Combining Fragment-Based Drug Design and X-ray Crystallography for the Development of Ethionamide Boosters as a New Strategy to Fight MDR-Tuberculosis

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Tuberculosis remains a major cause of mortality and morbidity, killing more than two million people each year. Although the combined use of three first-line antibiotics (isoniazid, rifampicin, and pyrazin-

amide) is effective for treating most patients, the rapid emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* stresses the need for alternative therapies. Ethionamide is one of the most frequently used drugs for the treatment of MDR tuberculosis. Consequently, as the number of MDR and XDR cases is growing worldwide, the importance of ethionamide is steadily increasing. However, ethionamide has a low therapeutic index, as the dose necessary to inhibit *M. tuberculosis* growth in patients (750 mg per day) generally causes serious adverse effects, such as gastrointestinal disorders, hepatitis, and various mental disturbances. Bioactivation of ethionamide by the mycobacterial monooxygenase EthA produces an ethionamide–NAD adduct that effectively inhibits the final target, InhA, an essential enzyme in cell-wall biosynthesis. The expression of *ethA*, in turn, is tightly controlled by the transcriptional repressor EthR. As such, genetically engineered *ethR* knockouts revealed a bacteria 25 times more sensitive to ethionamide, demonstrating that *M. tuberculosis* controls its sensitivity to ethionamide.

A new therapeutic concept emerged from this observation, leading to the design and development of first inhibitors of EthR.^[1–4] We demonstrated that the sensitivity of *M. tuberculosis* to ethionamide can be substantially increased in vitro and in vivo using such specific inhibitors. In the current study, we combined SPR assays, X-ray crystallography, in silico design, and medicinal chemistry for the rapid discovery and optimization of new structurally diverse EthR inhibitors based on two fragment-based drug design approaches. The design, synthesis, in vitro, and ex vivo activity of these compounds will be discussed.

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P383

New Stereoflexible Gateways to Hetero-2,5-diketopiperazines

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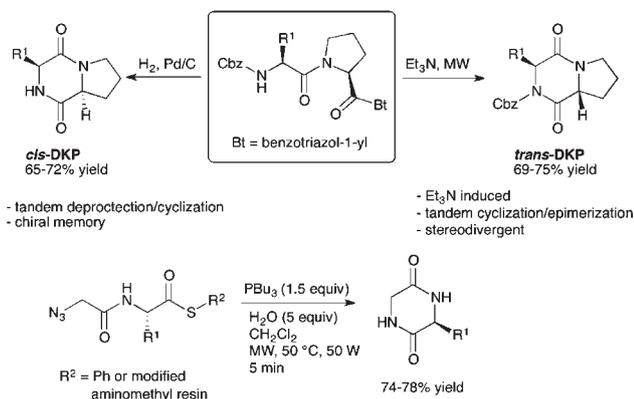
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Peptide and peptide-like structures have drawn great attention from the scientific community, especially in the area of medicinal chemistry. While small linear peptides have shown great promise as targets for bioactivity, they intrinsically suffer from disadvantages in their in vivo instability. In contrast, their cyclic analogues are usually associ-

ated with lower vulnerabilities to enzymatic degradation and tunable lipophilicity due to their conformation and structure. The smallest cyclic peptidoyl sequence, i.e., the 2,5-diketopiperazine (DKP) backbone, is a prominent fragment in a plethora of naturally occurring molecules. A wide range of biological activity has been reported so far: antibiotic, insecticidal, antimitotic, chemosensitizing, anti-HIV, etc. All these properties make DKPs substantial building blocks for the discovery of new leads and therapeutic agents.



As part of our ongoing research program dedicated to simple and efficient methods for the preparation of small cyclic peptides, we have now developed a series of protocols for the synthesis of optically pure DKPs. On the one hand we have designed a new versatile and stereoflexible strategy to provide either *cis*- or *trans*-configured DKPs starting from the same inexpensive L,L-dipeptidoyl benzotriazoles. A mechanistic investigation based on chiral HPLC, kinetics experiments, and computation afforded rationalization.^[1] On the other hand, as a complementary route, we also introduced a novel atom-economic and mild tandem deprotection–cyclization for the preparation of DKPs using and assessing a solid- and solution-phase Staudinger ligation, which allows rapid, convenient, and cost-effective cyclization.^[2]

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P384

Identification, Synthesis and SAR Investigation of a Series of Novel mGluR5 Negative Allosteric Modulators

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mGluR5 negative allosteric modulators (NAMs) have emerged as a novel and highly desirable class of compounds for the treatment of various CNS disorders including anxiety, Parkinson's disease levodopa-induced dyskinesia (PD-LID), depression, fragile X, migraine, and addiction/drug dependency. The growing number of clinical proof-of-concept studies anticipates the introduction of novel mGluR5 NAM as valuable therapeutic agents.

Through a HTS campaign, Addex has identified a structurally novel class of mGluR5 NAM. A lead optimization program focused on this series addressed the potency on the target, the selectivity and the optimization of ADME-PK properties. These efforts resulted in the identification of potent molecules (rat clone Ca^{++} flux, $\text{IC}_{50} < 50 \text{ nM}$) with a consistent SAR and SPR within the series.

P385

The Impact of the Molecular Lipophilicity Potential (MLP) on Molecular Docking

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The molecular lipophilicity potential (MLP)^[1] is a fragment-based molecular interaction field that has found broad application in computational chemistry and ligand-based drug design. Nowadays it is routinely used for 3D-QSAR as a lipophilic descriptor. Further enhancements showed that the MLP can also be successfully applied in target-based methods. A simple MLP-based adjustment of the definition of protein cavity hydrophobic areas allowed an overall gain of GOLD docking accuracy.^[2]

In order to unravel the impact of the MLP on other docking engines, the pre-calculated probe-based interaction potential maps of the docking tool AutoDock^[3] were modified by introducing an additional MLP-based factor. Such a factor aims at a better description of hydrophobicity in the protein pockets and properly guides ligand hydrophobic moieties toward favorable areas. Docking results based on such MLP-based maps showed a higher success rate in re-docking

simulations than docking with the unmodified maps. Moreover, an MLP-based score was developed, demonstrating high efficacy in re-scoring and re-ranking docking results.

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Structural, Electronic and Spectral Properties and Keto–Enol Tautomerism of N-Substituted 3-Amino-5-hydroxy-4-phenyl-1H-pyrazolo-1-carboxamides with Antibacterial Activity

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Pyrazole derivatives exhibit anti-inflammatory, analgesic, anti-fever, antidiabetic, sedative and hypnotic, anticoagulative, antiviral, antibacterial, and anticancer properties.^[1] Searching for novel pharmacologically active compounds, we obtained N-substituted 3-amino-5-hydroxy-4-phenyl-1H-pyrazolo-1-carboxamides with antibacterial activity.^[1] As pharmacological properties of chemical compounds are clearly connected with their structure, the elucidation of structural parameters of novel compounds is essential to rationalize their activity.

The aim of this study is the computational determination of structural, electronic, and spectral (¹H and ¹³C NMR chemical shifts, vibrational frequencies) parameters of novel compounds, verification of the results by comparison with available experimental data, and linking them to pharmacological activity. The methods applied involved Hartree–Fock and density functional B3LYP with the 6-31G(d,p) basis set of Gaussian09. The keto–enol tautomerism of the pyrazolinone moiety is also studied, as we have previously found that this phenomenon determines pharmacological activity in similar systems.^[2,3] In the gas phase and crystalline state all molecules exist in keto

form, while in DMSO and chloroform solutions a small amount of enol form is observed. The results obtained will be used for future docking and QSAR studies.

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Searching for the Dimerization Interface of the D₂R–mGluR5 Heterodimer in Different Conformational States

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The human mGluR5–D₂R heterodimer, a complex of the metabotropic glutamate type 5 receptor and the dopamine D₂ receptor, is a promising drug target for the treatment of schizophrenia, tackling two main causes of this disease: dopaminergic and glutamatergic. To apply structure-based drug design techniques to search for better antipsychotics, exerting their action through the mGluR5–D₂R heterodimer, it is necessary to construct 3D models of this receptor complex.

The aim of this work is the search for the dimerization interface of the human mGluR5–D₂R heterodimer in different conformational states of both protomers (active or inactive). The appropriate protomer models are constructed with Modeller v.9.10. To determine the most probable dimerization interface, we apply our multi-component protocol which we demonstrated to be highly successful for modeling GPCR dimers. The method applies protein–protein docking with Rosetta software to obtain populations of dimers as present in membranes with all possible interfaces (49 interfaces for a heterodimer), followed by a consensus scoring procedure according to 1) Rosetta score, 2) surface of the dimer interface, 3) polar contribution to the dimer interface, 4) fractal dimension of the dimer interface,^[1] 5) evolutionary conservation score, 6) shape complementarity, and 7) electrostatic complementarity. The best

models are minimized, and the whole cycle is iteratively repeated until the results converge to a consistent dimer formation. The protocol enabled us to determine that the most probable dimerization interfaces for the human mGluR5–D₂R heterodimer is formed by TM4–TM5 or TM5–TM6 regions of both receptors which is consistent with available experimental data.

Acknowledgments: This study was performed during the postdoctoral stay of A.A.K. at the University of Regensburg, funded by the Deutscher Akademischer Austauschdienst (DAAD). Part of the calculations was performed under a computational grant by the Interdisciplinary Center for Mathematical and Computational Modelling (ICM), Warsaw, Poland; grant number G30-18.

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Assessment of Protein–Protein Docking Tools for Modeling Dimers of G-Protein-Coupled Receptors

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Recently, the process of dimerization of G-protein-coupled receptors (GPCRs), one of the most important groups of drug targets, has gathered particular attention, as it opens new possibilities for GPCR-oriented drug discovery. To study the mechanisms and dynamics that govern GPCR oligomerization, it is essential to understand the dynamic process of receptor–receptor association and to identify regions that are suitable for selective drug binding. Because X-ray structures are currently available for only two physiologically relevant GPCR dimers (the chemokine CXCR4 homodimer and the κ opioid receptor homodimer),^[1] various computational techniques have been applied for modeling GPCR dimers based on sequence analysis, protein–protein docking algorithms, or molecular dynamics techniques.^[2]

The aim of this work is to perform a systematic analysis of the applicability of widely used protein–protein docking servers for modeling GPCR dimers. The following servers are studied: ZDOCK, ClusPro, HEX, GRAMM-X, PatchDock, SymmDock, and HADDOCK. The research, aimed to reconstruct GPCR complexes in a blind docking procedure, is performed on two rhodopsin homodimers (PDB IDs: 1N3M and 2I37), the chemokine CXCR4 homodimer (PDB ID: 3OE9), the κ opioid receptor homodimer (PDB ID: 4DJH), and the published mGluR2–5-HT_{2A} heterodimer model. The obtained complexes are scored according to several criteria, including CAPRI criteria. For both rhodopsin dimers

no satisfactory results are obtained by any of the studied servers, whereas for the κ opioid receptor dimer, high-quality models are constructed by HADDOCK and ClusPro (in symmetrical docking), for the CXCR4 homodimer, by HADDOCK, and for the mGluR2–5-HT_{2A} dimer, by GRAMM-X. In summary, our study indicates that currently available protein–protein docking software has limitations when modeling complexes of transmembrane proteins, in particular GPCR dimers.^[3] Hence, there is an urgent need to elaborate modeling protocols considering membrane-specific characteristics of such protein complexes which is the focus of our research efforts.

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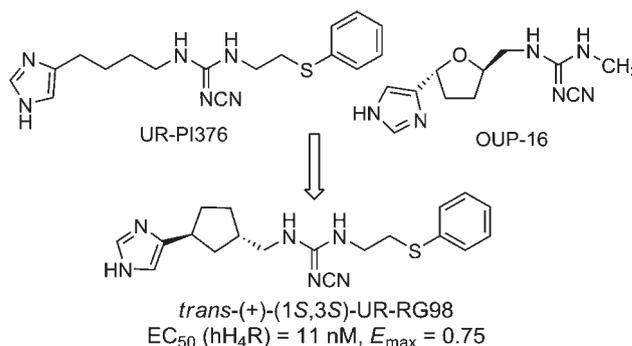
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trans-(+)-(1S,3S)-UR-RG98: Synthesis, Absolute Configuration and Pharmacological Characterization of a Highly Potent and Selective Histamine H₄ Receptor Agonist

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The human histamine H₄ receptor (hH₄R) is suggested to play a crucial role in immunological and inflammatory processes.^[1] To explore the (patho)physiological function of the hH₄R, selective ligands—antagonists as well as agonists—are needed.



We recently reported the synthesis and structure–activity relationships of conformationally constrained cyanoguanidines derived from UR-PI376^[2] and OUP-16.^[3] Among a series of imidazolylcyclopentylmethylcyanoguanidines, *trans*-(+)-UR-RG98 was the most potent and selective H₄R agonist, with an EC₅₀ of 11 nM, a greater than 100-fold selectivity for the H₄R over the H₃R and negligible activities at the other HR subtypes in the [³⁵S]GTPγ-S assay.^[4] By contrast, the optical antipode *trans*-(–)-UR-RG98 proved to be an antagonist at the H₄R.

To determine the absolute configuration of the title compound, a stereoselective synthesis was developed, which selectively provided one *cis* and one *trans* isomer. The 3D structure of intermediates was resolved by X-ray analysis. Comparison of chromatograms (HPLC) in combination with UV and CD detection of these products with those of the investigated isomers allowed unequivocal assignment of the absolute configuration of all stereoisomers and the identification of *trans*-(+)-(1*S*,3*S*)-UR-RG98 as the eutomer.

In addition to functional [³⁵S]GTPγ-S assays, radioligand binding assays were performed, and the pharmacological data for *trans*-(+)-(1*S*,3*S*)-UR-RG98 and its stereoisomers were validated in luciferase reporter gene assays at human (h), mouse (m), and rat (r) H₄R using HEK293-SF-H₄R-His₆-CRE-Luc cells expressing the respective H₄R species orthologue. The results proved that *trans*-(+)-(1*S*,3*S*)-UR-RG98 is a versatile pharmacological tool for the study of the H₄R.

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Characterization of Complexes of Nucleoside 5'-Thiophosphate Analogues with Zinc Ions

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Ectonucleotide pyrophosphatase 1 (NPP1) regulates pathologic calcification principally via PPI generation from nucleoside triphosphates in tissues. NPP1 contains zinc ion at the catalytic site. Therefore, inhibition of NPPs by Zn^{II} chelators is highly desirable. As the Zn^{II} ion is thiophilic, we targeted nucleoside 5'-thiophosphate analogues as potential biocompatible Zn^{II} chelators. We synthesized nucleoside 5'-thiophosphate analogues such as GDP-β-S, ADP-β-S and AP₃(β-S)A and characterized their complexes with zinc ions. We studied their geometry and acidity and stability constants. We established the stability constants of the complexes of thiophosphate analogues with zinc ions by applying potentiometric titrations and HYPERQUAD software refinement. We evaluated the effect of the thiophosphate versus phosphate moiety on the stability of nucleotide–Zn^{II} complex and found that the thiophosphate moiety enhanced the complex stability. ATP-α,β-CH₂-γ-S forms the most stable complex with Zn^{II}

(logK 6.50), being ~0.8 and ~1.1 log units more stable than ATP-γ-S-Zn^{II} and ATP-Zn^{II}, due to its electron-donating methylene group. ATP-α,β-CH₂-γ-S, ADP-β-S, and GDP-β-S complexes with Zn^{II} were 1.1–1.3 log units more stable than the parent nucleotide complexes. Likewise, stability constant of the complex AP₃(β-S)A with zinc was ~0.5 log units higher than that of AP₃A. We also found that the terminal thiophosphate analogues were more acidic than their parent nucleotides. In addition, we investigated the coordination sites of Zn^{II} ions in complex with thiophosphate analogues by ¹H and ³¹P NMR. We found that Zn^{II} coordination sites in nucleoside 5'-thiophosphate analogues are N7 and the two terminal phosphates. Titration of nucleoside 5'-thiophosphate analogues with Zn^{II} monitored by ³¹P NMR, we observed upfield shifts unlike the downfield shifts for their parent nucleotide–Zn^{II} complexes. In conclusion, a thiophosphate group at a terminal position resulted in increased stability constants of the corresponding Zn complexes as compared with those of the corresponding complexes of dinucleotides. ATP-α,β-CH₂-γ-S was found to be the most stable Zn chelator.

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Multi-component Protocol for Modeling Dimers of G-Protein-Coupled Receptors

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Classical structure-based drug design techniques using G-protein-coupled receptors (GPCRs) as targets focus nearly exclusively on binding at the orthosteric site of a single receptor. However, more and more experimental (AFM, FRET, BRET, cysteine cross-linking receptor chimera studies)^[1] and computational (molecular dynamics, protein–protein docking, sequence-based methods)^[2] evidence indicates the existence of physiologically relevant GPCR dimers or oligomers. Targeting these complexes selectively or designing small molecules that affect receptor–receptor interactions might provide new opportunities for novel drug discovery.

In this scenario, the aim of this work is the elaboration of an efficient multi-component protocol for modeling GPCR dimers. The method is tested on four homodimers for which 3D structures are available: rhodopsin homodimer (semiempirical model, PDB ID: 1N3M), κ opioid receptor homodimer (X-ray structure, PDB ID: 4DJH), chemokine CXCR4 homodimer (X-ray structure, PDB ID: 3OE9) and β₂ adrenergic receptor homodimer (model). The method involves application of protein–protein docking with Rosetta software to obtain populations of dimers as present in membranes with all main possible interfaces (28 interfaces for a homodimer) followed by a

consensus scoring procedure according to 1) Rosetta score, 2) surface of dimer interface, 3) polar contribution to dimer interface, 4) fractal dimension of dimer interface,^[3] 5) evolutionary conservation score, 6) shape complementarity, and 7) electrostatic complementarity (including our novel approach to electrostatic complementarity; see the accompanying poster). The best models are minimized, and the whole cycle is iteratively repeated until the results converge to a consistent dimer formation. Strikingly, the protocol enabled us to reproduce structures of rhodopsin, κ opioid, and CXCR4 receptor homodimers and to build a β_2 homodimer model consistent with experimental data.

Acknowledgments: This study was partially performed during the postdoctoral stays of A.A.K. at the GRIB, Barcelona, funded by the Foundation for Polish Science (FNP, Kolumb outgoing fellowship) and at the University of Regensburg, funded by the Deutscher Akademischer Austauschdienst (DAAD). This work was also funded by the Spanish Ministerio de Ciencia e Innovación (SAF2009-13609-C04), and Fundación La Marató de TV3 (Ref.-No. 091010). Part of calculations was performed under a computational grant by the Interdisciplinary Center for Mathematical and Computational Modelling (ICM), Warsaw, Poland; grant number G30-18.

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Inhibitors of the *Plasmodium falciparum* Protein Kinase CDPK1 as Potential Novel Antimalarial Agents

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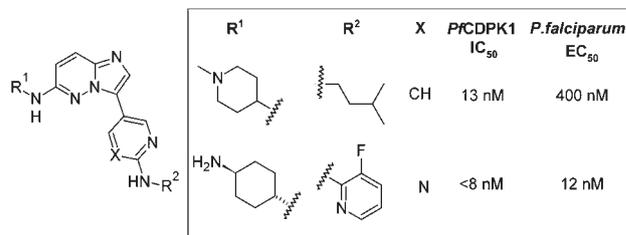
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Malaria is one of the most prevalent infectious diseases of the developing world. In excess of three billion people are at risk, and it currently leads to the deaths of almost one million people each year, with the majority of these being in sub-Saharan Africa and children under 5.^[1] Resistance to existing antimalarial drugs is widespread, and therefore new therapeutic approaches are urgently needed.

Calcium-dependent protein kinases (CDPKs) are directly regulated by Ca^{2+} and are found in plants and organisms in the alveolate lineage,^[2] but are absent in humans. They are present in *Apicomplexan* parasites including *Plasmodium falciparum*, the causative agent of

the most severe form of malaria. *P. falciparum* calcium-dependent protein kinase 1 (PfCDPK1) is encoded by an essential gene and is responsible for phosphorylation of components of the molecular motor that drive parasite invasion of red blood cells.^[3] If this invasion process can be prevented the parasite lifecycle is broken, leading the parasites to die and thus potentially allowing the infection to be cleared. PfCDPK1 therefore represents a novel target for the potential treatment of malaria and offers promise for achieving selectivity over the kinases of the human host.

A high-throughput screen of our compound collection and a subsequent medicinal chemistry programme has yielded compounds which show low nanomolar binding affinity to the enzyme, and promising activity against the *P. falciparum* parasite in a cellular assay. Concomitant optimisation of their in vitro ADMET properties has afforded compounds with suitable PK profiles for oral dosing in vivo in a *P. berghei* murine model of malaria, and efficacy data from this model will be presented. A structure-based design approach using a homology model of PfCDPK1 has allowed compounds with increased affinity against the enzyme and improved cell potency against *P. falciparum* to be obtained. Screening of these compounds against a panel of human protein kinases has demonstrated promising selectivity profiles, and they also show selectivity for CDPK1 over related malarial CDPKs.



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Synthesis of Aerucyclamide Analogues with Antimalarial or Antitrypanosomal Activity

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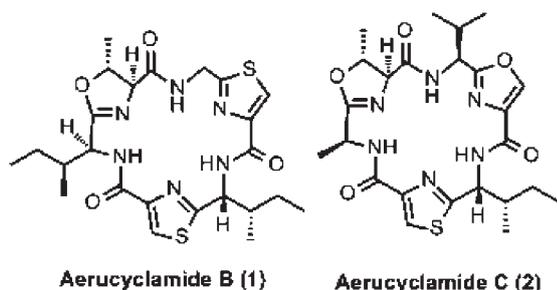
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Various bioactive cyanobacterial compounds exhibit unique structural features like cyclic peptides containing azole heterocycles.^[1] Aerucyclamides are examples of azole cyclopeptides, isolated in 2008 by Gademann and co-workers from the toxic freshwater cyanobacterium *Microcystis aeruginosa* PCC 7806.^[2] Aerucyclamide B (**1**) displays potent and selective antiplasmodial activity against *P. falciparum* K1, and aerucyclamide C (**2**) is the most active of them against *T. brucei rhodesiense*.

According to the World Health Organization 2011 report, there were an estimated 216 million episodes and 655000 deaths by malaria in 2010.^[3] Various antimalarial drugs are presently used for the treatment of this tropical disease, but unfortunately the rapid spread of resistance has seriously compromised their efficacy.^[4]

As part of our search for candidates for new antiparasitic drugs, we embarked on the synthesis and biological evaluation of macrocyclic analogues of aerucyclamides. In the present work we present the preparation of key fragments and macrocycle analogues and their biological evaluation against *P. falciparum* K1 and *T. brucei*. Some of the obtained products show enhanced activity compared with aerucyclamides.



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Synthesis and Biological Evaluation of 2-Aminopurine Derivatives as Heat Shock Protein 90 Inhibitors

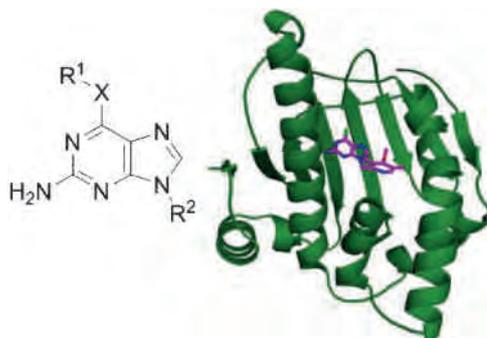
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Heat shock protein 90 (Hsp90) is a molecular chaperone involved in the correct folding, stabilization, and activation of various proteins by forming multimeric protein complexes with ADP/ATP, its co-chaperones, and many client proteins which are essential in cell activation and proliferation. Because Hsp90 also stabilizes a number of oncogenic proteins that are mutated or overexpressed in tumor cells and drive cancer cell growth, a number of Hsp90 inhibitors are currently undergoing clinical evaluation in cancer patients.^[1] Among them, we are interested in the purine-based inhibitors such as PU-H71, CUDC-305, MPC-3100, and BIIB021.

We synthesized a series of 2-aminopurine derivatives containing amino or oxy modifications at the 6-position to introduce an additional pharmacophore. The prepared compounds showed good binding affinity to Hsp90 in ITC assays and exhibited good antiproliferative activities against MCF-7 and HCT-116 cancer cell lines. X-ray crystallographic data of the ligand–Hsp90 complex showed the inhibitors bind in the ATPase site of the Hsp90 N-terminal domain with an L-shaped conformation. The aryl ring at position N9 showed π – π stacking interactions with Phe138 in the hydrophobic pocket by reserving the conformation of outer helix.



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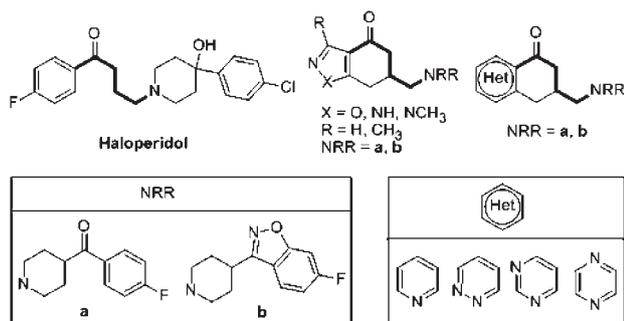
Conformationally Constrained Aminobutyrophenones. New Heterocyclic Analogues of Haloperidol as Potential Atypical Antipsychotics

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The newer generation of treatments for schizophrenia, referred to as atypical antipsychotics, add to the blockade of dopamine receptors, a potent activity at serotonin receptors. It is thought that 5-HT_{2A} antagonism, together with relatively weaker dopamine antagonism, are principal features that differentiate the side effect profile of atypical antipsychotics, such as clozapine, from the first generation of treatments.^[1] Although the novel atypical antipsychotics olanzapine, risperidone, and quetiapine have brought about improvements in toleration and negative symptomatology, chronic treatment may lead to unwanted weight gain, blood dyscrasias, and motor dysfunctions, such as extra-pyramidal side effects (EPS) and tardive dyskinesia (TD). These side effects may be linked to drug-dependent affinity for other receptors. The search in our group continues for new atypical antipsychotics that would be more efficacious and would have fewer side effects than currently available treatments.^[2]

Because antidopaminergic therapies continue in the first line of treatment for schizophrenia, herein we describe our recent efforts to discover novel templates in the area of selective dual 5-HT_{2A}/D₂ antagonists for potential use as treatments for schizophrenia. In these compounds a typical bioisosteric replacement of the benzene in the aminobutyrophenone pharmacophore by a five- or six-membered heterocycle containing at least one heteroatom has been applied.



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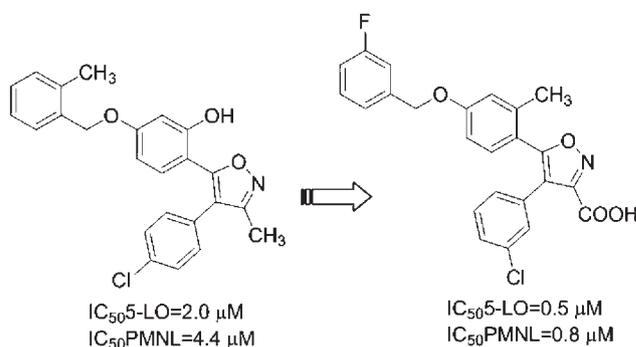
P396

New 4,5-Diarylisoazole Derivatives as Novel Inhibitors of Human 5-Lipoxygenase (5-LO)

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Human 5-lipoxygenase (5-LO) catalyzes the early step in the biosynthesis of leukotrienes (LTs) from arachidonic acid. LTs are mediators of various pathological conditions including inflammation and allergic reactions, and it has recently been demonstrated that the 5-LO pathway is also associated with atherosclerosis and cancer. Therefore, 5-LO inhibitors have been extensively studied as potential candidates for novel therapies for inflammatory allergic, cardiovascular, and respiratory diseases including asthma. However, only one compound, an iron ligand inhibitor zileuton, has reached the market. In the present work, we report the synthesis, 5-LO inhibitory potential, and docking calculations of new 4,5-diarylisoazole derivatives as novel inhibitors of 5-LO for the suppression of LT biosynthesis.



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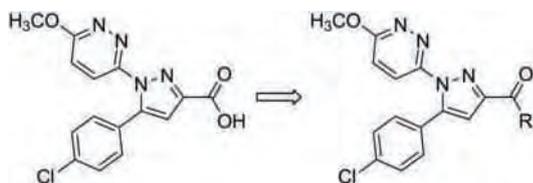
P397

Novel 1,5-Diarylpyrazole Derivatives as Potent Antiplatelet Agents against Arachidonic Acid-Induced Platelet Aggregation

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Platelet aggregation inhibitors have great potential for prevention or treatment of ischemic diseases such as cardiovascular thrombosis, which occurs after aggregation of platelets due to endothelial damage caused by a ruptured atherosclerotic plaque, thereby resulting in vascular occlusion. The most common of these antiplatelet agents include aspirin, clopidogrel, and integrin α Ib β 3 antagonists. However, limitations of current antiplatelet agents, including weak inhibition of platelet function, slow onset of action, and increased risk of bleeding still require the development of new molecules with potent antiplatelet activity. In the course of our studies aimed at obtaining new human platelet aggregation inhibitors, we synthesized numerous amide and ester derivatives of 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylic acid and tested their in vitro antiplatelet activity. Among them, amide derivatives showed potent antiplatelet activity against arachidonic acid-induced platelet aggregation with IC_{50} values in the range of 0.04–0.6 μ M. The most potent derivatives were those with small basic amide side chains such as piperidine and morpholine.



R	IC_{50} [μ M]
morpholine	0.04
piperidine	0.08
4-Cl-aniline	0.60
4-OMe-aniline	0.40
4-aminopyridine	0.10

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P398

Hsp90 Inhibitors for Oral Treatment in Oncology

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Molecular chaperones are proteins that play a key role in the conformational maturation, stability, and function of client proteins within the cell.^[1,2] Many of the client proteins of Hsp90^[3-6] are involved in signal transduction, cell-cycle regulation, and apoptosis, including kinases, transcription factors, and hormone receptors. The dysregulation of pathways involving these proteins are commonly associated with cancer pathology.^[7] Hsp90 has attracted considerable interest as a therapeutic target for anticancer drugs since it was shown that both geldanamycin^[8] and radicicol^[9] are able to inhibit Hsp90 function by binding to an ATP binding pocket in the N-terminal domain of the protein. Based on encouraging preclinical data, several derivatives of these natural product inhibitors such as 17-AAG, 17-DMAG, and IPI-504 have entered clinical studies.^[10] However, due to several limitations of these compounds, significant efforts have been made to identify small-molecule inhibitors. Indeed, several compounds of different chemical classes have been disclosed so far, like ganetespib (STA-9090),^[11] NVP-AUY922,^[12] AT-13387,^[13] Debio-0932 (CUDC-305),^[14] and PU-H71,^[15] which have entered trials in various phases of clinical development.

We identified compounds using different approaches. HTS hits from the resorcinol class were co-crystallized with Hsp90, and the elucidated binding mode to the N-terminal ATPase pocket was exploited for subsequent optimization of potency and oral bioavailability. Here we present how the SAR evolved from initial hits with respect to potency, physicochemical properties, and pharmacokinetic properties toward a clinical candidate with improved oral bioavailability and in vivo potency.

We also show how we identified potential back-up compounds using a fragment-based approach. Fragments were selected by docking, tested in a biochemical assay, and the confirmed hits were crystallized. Information gained from X-ray structures of each fragment was used to drive the fragment evolution process. Optimization of these micromolar binders from different structural classes resulted in inhibitors with significantly improved affinity. Further optimization toward drug-like molecules yielded compounds which demonstrate antiproliferative effects across a spectrum of cancer cell lines and down-regulate Hsp90 clients. Moreover, these derivatives show significant tumor growth inhibition in vivo after oral administration.

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kNN Classification Models to Predict BBB Penetration Using Sampling Techniques and Imbalanced Dataset

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Blood–brain barrier (BBB) penetration is an important ADME property, considered in drug design, and there are several computational models available for its prediction. However, most of these approaches are based on imbalanced datasets, and therefore the resultant models are biased. Herein we report for the first time a promising approach that helps overcome the imbalanced nature of the BBB dataset of 335 BBB– and 1302 BBB+ compounds. We used 1) synthetic minority over-sampling technique (SMOTE) for re-sampling, 2) genetic algorithm (GA) for variable selection, and 3) k-nearest neighbor (kNN) for classification. From our analyses we observe that the models 1) have excellent predictive ability, 2) are robust, and 3) are easily interpretable. Finally, we infer that imbalance in BBB penetration data can be effectively addressed using re-sampling techniques for the generation stable prediction models.

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P400

Valeriana wallichii Roots as a Source for New Drugs with Antileishmanial Activity

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Leishmaniasis is one of the most common parasitic diseases, affecting more than 12 million people worldwide and causing an estimated 70000 deaths per year. Annually about two million people, mostly in developing countries, get infected with one of the 17 different species of protozoa of the genus *Leishmania*.^[1,2] The absence of a vaccine, the growing resistance against widely used antileishmanial agents, and the significant toxicity of drugs in use demonstrate the urgent need of new antileishmanial substances. In traditional Indian folk medicine *Valeriana wallichii* is well known and used, e.g. in the treatment of insomnia, epilepsy, or anxiety.^[3] Recently the discovery of activity against *Leishmania major* promastigotes in a fraction of a chloroform extract of *V. wallichii* roots was reported by Ghosh et al.^[4] To discover the compound(s) responsible for the antiprotozoal activity, a chloroform extract of *V. wallichii* roots from India was prepared and subjected to an activity-guided fractionation by preparative HPLC and column chromatography. IC₅₀ values were determined by AlamarBlue™ assay on *L. major* promastigotes. Multiple fractionation resulted in several fractions with elevated antileishmanial activity with IC₅₀ values ranging from 0.7 to 12 µg mL⁻¹. To elucidate the structures of the molecules contained in these fractions, NMR spectroscopy and ESIMS were applied. One molecule could be identified as the main component of a fraction, with an IC₅₀ value of 11.8 µg mL⁻¹. This compound and structurally related derivatives were synthesized to confirm its antileishmanial potential. Further fractionation of the extract, purification, and identification of possible active compounds is still ongoing.

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P401

[³H]UR-DE257—A New Tritium-Labeled Human Histamine H₂ Receptor Antagonist

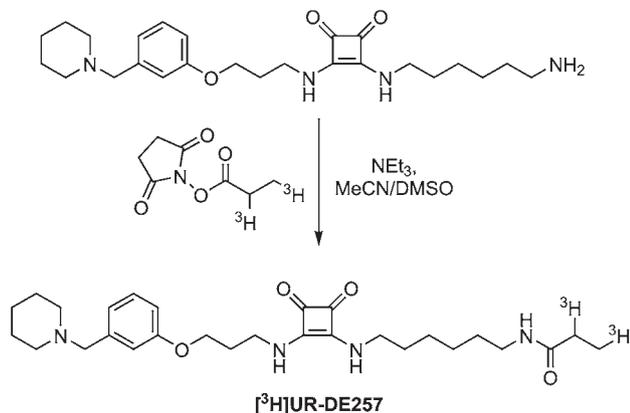
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As there has been a lack of high-affinity radioligands for the investigation of the histamine H₂ receptor (H₂R), we synthesized and characterized *N*-[6-(3,4-dioxo-2-[3-(3-(piperidin-1-ylmethyl)phenoxy)propylamino]cyclobut-1-enylamino)hexyl]-[2,3-³H]propionamide ([³H]UR-DE257) as an easily accessible tritium-labeled human histamine H₂ receptor (hH₂R) antagonist ($K_b=38$ nM, neutral antagonism in the GTPase assay) derived from BMY25368.^[1]

Acylation of the amine precursor with succinimidyl [2,3-³H]propionate allows the convenient preparation of a tritium-labeled radioligand with a high specific activity (63 Ci/mmol). This radioligand binds with sufficiently high affinity (from saturation binding: $K_b=31$ nM; kinetic experiments: $K_b=20$ nM) and selectivity for hH₂R over the other HR subtypes. Potencies, intrinsic activities and selectivities of the unlabeled form, UR-DE257, were determined in functional assays, for example, in GTPase assays using membrane preparations of Sf9 insect cells, expressing the respective hH₂R subtype (hH₁R: $K_b > 10000$ nM, hH₂R: $K_b=848$ nM, inverse agonism, $\alpha=-0.46$, hH₃R: $K_b > 5000$ nM).

The title compound is shown to be a valuable pharmacological tool for the determination of hH₂R affinities. Because of the practicability and low costs of this labeling strategy,^[2,3] [³H]UR-DE257 is a highly attractive alternative to [³H]tiotidine, which is not commercially available at the moment and shows a high degree of unspecific binding. [³H]UR-DE257 has also advantages over radiolabeled [¹²⁵I]-iodoaminopotentidine due to the considerably longer half-life and easier handling with respect to safety precautions.



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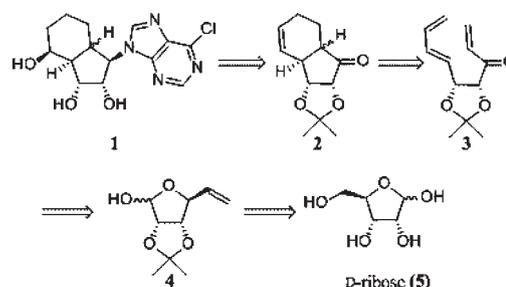
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Synthetic Studies of Carbobicyclic Nucleosides

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The replacement of the furanose ring by a cyclopentane in nucleosides generates a series of analogues known as carbobicyclic nucleosides, which show increased chemical and enzymatic stability because the glycosidic bond is absent.^[1] However, the loss of such glycosidic bond leads to a significant change in conformation due to the absence of anomeric effect and gauche effect that help maintain the furanose ring in either 3'-endo (north) or 2'-endo (south) conformation in conventional nucleosides.^[2] Fusing a carbocyclic ring to a cyclopentane should be able to lock the embedded cyclopentane ring into a conformation similar to that in conventional nucleosides.^[3] Herein, the synthetic study of conformation constrained carbobicyclic nucleosides, a novel bicycle[4.3.0]nonane system, will be reported. Nucleoside **1** could be derived from ketone **2**, which would be assembled from enone **3** via an intramolecular Diels–Alder reaction as the key step. Enone **3** would be accessed from D-ribose (**5**).



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P403

Study on Signal Transduction Pathway Responsible for the Insulin Mimetic Activity of Neohesperidose

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Introduction: Insulin mimetic activity of some flavonoids and its glycoside had been reported previously. These compounds are known to have antihyperglycemic effect in vivo and are thought effective in prevention or treatment of diabetes mellitus. We have previously focused and studied the insulin mimetic activity of kaempferol 3-*O*-neohesperidoside, which shows strong activity in nanomolar concentration,^[1] and as a result of a structure–activity relationship (SAR) study, we showed that the disaccharide **1** is the main structure responsible for insulin mimetic activity.^[2]

Insulin's activity is exerted through a signaling pathway beginning from insulin receptor. This receptor on cellular membrane is first phosphorylated, which pass its signal through IRS-1, PI3K, and PKB, then glucose transporter 4 (GLUT4) localized at endoplasmic reticulum migrates to the cell surface and absorbs extracellular glucose into the cell (Figure 1a). However, it is not known how **1** induces its activity. In this investigation, we studied the action of **1** against L6 skeletal cell and 3T3-L1 adipocyte cell to reveal signaling pathway of insulin mimetic activity.

Results and Discussion: We detected the phosphorylation of insulin receptor and IRS-1 after stimulation of differentiated L6 cell and 3T3-L1 cell with insulin or **1**. IRS-1 is one of the intracellular substrate and its phosphorylation is critical in insulin signaling. After cell lysate preparation, immunoprecipitation, SDS-PAGE and Western blotting were conducted. Also, PI3K was blocked with LY29004 (PI3K inhibitor) to judge whether insulin mimetic activity of **1** need PI3K. The result of Western blotting is shown in Figure 1b. Insulin receptor and IRS-1 phosphorylation were not detected after stimulation of **1**, suggesting **1** enhances muscle glucose uptake independent of insulin receptor and IRS-1. We are now examining the effect of LY29004.

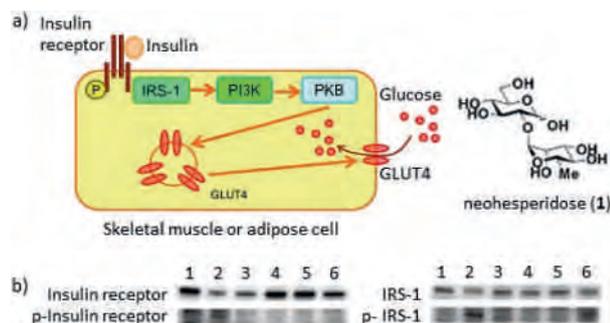


Figure 1. a) The insulin signaling pathway and the structure of neohesperidose (**1**). b) Western blots: detection of phosphorylated insulin receptor (left) and detection of phosphorylated IRS-1 (right). Lane 1: without stimulation; lane 2: with 100 nM insulin for 10 min; lanes 3–6: 0.1 nM neohesperidose for 1, 2, 3, 4h, respectively.

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P404

Antimicrobial Characterization and Biocompatibility of a Chitosan/Dextran-Based Hydrogel for Surgical Use

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A chitosan/dextran-based (CD) hydrogel has been developed as a post-surgical aid in endoscopic sinus surgeries (ESS) in vivo in both animals and humans. The CD hydrogel significantly reduced the number of adhesions as well as exhibiting excellent haemostatic, mucoadhesive, and antimicrobial properties.^[1–4] Antimicrobial action of the hydrogel in vitro against both Gram-negative and Gram-positive bacteria was investigated. The hydrogel's antimicrobial mechanism of action was investigated.^[5] Mutagenicity of the hydrogel was assessed using the Ames test and found to be non-mutagenic. The study is currently testing the cytotoxic impact of this hydrogel in vivo on human macrophages, nasopharyngeal epithelial, and fibroblast. Next, we will examine the pro-inflammatory response of the gel in vitro, and its influence on the process of wound healing.

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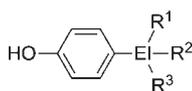
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Hydrophobic Parameters, Substituent Constants and Estrogenic Activities of Silicon and Germanium-Containing Phenols

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Silicon and germanium-containing compounds have drawn the attention in the drug discovery because of their inherent properties different from the carbon analogues. However, the characteristics of silicon and germanium-containing substituents have not been revealed sufficiently. We have synthesized various trialkylsilyl- and trialkylgermylphenols and investigated characteristics of silyl and germyl functional groups as hydrophobic substituents of bioactive compounds. Trialkylsilyl- and trialkylgermylphenols exhibited higher hydrophobicity than the corresponding carbon analogues with difference of $\log P$ value of 0.5–0.7. There was no significant difference between hydrophobicity of silylphenols and that of corresponding germylphenols. The trialkylsilyl- and trialkylgermylphenols exhibited smaller pK_a values than the corresponding carbon analogue, indicating that these silyl and germyl functional groups have negative substituent constant, contrasting with carbon functional groups that bearing positive substituent constant. We also found trialkylsilyl- and trialkylgermylphenols exhibited potent estrogenic activity toward estrogen-dependent MCF-7 cell proliferation exceeding that of corresponding carbon analogues. These properties are meaningful for drug discovery based on the heavier 14 group elements, and the detailed parameters and the structure–activity relationship will be discussed.



EI = C, Si or Ge
 $R^1, R^2, R^3 = \text{Me, Et, } n\text{-Pr, Ph etc.}$

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3D-QSAR and Virtual Docking Study of Agonists and Antagonists at Imidazoline-1 Receptor

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The imidazoline-1 receptors (I_1 -IR) agonists are drugs used in treatment of high blood pressure and hyperglycemia. The I_1 -IR protein structures have not yet been determined to date, but nischarin protein (NISCH; UniProt ID: Q9Y2I1) – strong I_1 -IR candidate has been cloned.^[1] Since in vivo pharmacological effects of the I_1 -IR agonists, I_1 -IR-partial agonists and I_1 -IR antagonists are very diverse, our main aim was to define the most important pharmacophores responsible for I_1 -IR agonistic activity, select pharmacophores related to I_1 -IR antagonistic activity, develop specific 3D quantitative structure–activity relationship (3D-QSAR) models for the prediction of I_1 -IR agonistic and I_1 -IR antagonistic activity, and examine specific binding site for I_1 -IR agonists and for I_1 -IR antagonists on I_1 -IR.

The 3D-QSAR and virtual docking studies of 29 I_1 -IR ligands (agonists, partial agonists and antagonists) were carried out on I_1 -IR receptors binding affinities. Recently developed highly selective I_1 -IR ligands, such as S23515, S23757, LNP509, LNP906, and LNP911, were included in the 3D-QSAR and docking study of the I_1 -IR. Initial 3D-QSAR study was performed on the 14 I_1 -IR agonists and the 3D-QSAR (I_1 -IR agonists) model relating 58 variables, with three significant components ($A=3$), R^2 : 0.95, Q^2 (Y): 0.69, r^2 observed versus predicted: 0.977, RMSEE: 0.325, and RMSEP: 0.401, was created. The following 3D-QSAR study of the 13 I_1 -IR antagonists resulted in development of the 3D-QSAR (I_1 -IR antagonists) model relating 104 variables, with two significant components ($A=2$), R^2 : 0.88, and Q^2 (Y): 0.78, r^2 observed versus predicted: 0.916, RMSEE: 0.579, and RMSEP: 0.465.

Developed pharmacophores and the most significant variables of the 3D-QSAR (I_1 -IR agonists) and 3D-QSAR (I_1 -IR antagonists) models were compared. The presented 3D-QSAR (I_1 -IR agonists) and 3D-QSAR (I_1 -IR antagonists) modeling is first pharmacophore studies of I_1 -IR agonists and I_1 -IR antagonists. The nischarin domains mapping was performed by use of the Informational Spectrum method (ISM).^[2] The docking study of the 29 I_1 -IR ligands was performed on the optimized binding site of the nischarin protein. Different binding sites in the active domain of the nischarine were detected for the I_1 -IR agonists and the I_1 -IR antagonists. As a corollary, the specific binding sites, predicted by ISM bioinformatics method and further precisely defined by virtual docking, for I_1 -IR agonists and for I_1 -IR antagonists on the nischarin protein sequence, together with the created 3D-QSAR (I_1 -IR agonists) and 3D-QSAR (I_1 -IR antagonists) models provide reliable system for evaluation of I_1 -IR agonistic and I_1 -IR antagonistic activity of the related imidazoline ligands.

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P407

Native Chemical Ligation of Hydrolysis-Resistant 3'-Peptidyl-tRNA Mimics Conferring Resistance to Macrolide Antibiotics

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RNA-peptide conjugates are highly requested compounds for structural and functional studies of the ribosomal elongation cycle.^[1] The ribosomal elongation of the peptide chain takes place at the peptidyl transferase center (PTC) of the ribosome. Thereby, the growing peptide chain has to pass through the ribosomal exit tunnel before it can leave the ribosome. Macrolide antibiotics bind at the entrance of this tunnel and thus prevent elongation of the peptide chain.^[2] However, bacteria can show resistance by translating short specific peptides that are highly conserved in their sequence. It is hypothesized that the macrolide interacts in a specific manner with the resistance peptide and thus is expelled from the ribosomal exit tunnel so that the ribosome is available for protein synthesis again.

Hydrolysis-resistant 3'-peptidyl-RNA conjugates that mimic acylated tRNA termini represent a remarkable synthetic challenge, in particular if they contain amino acids with complex side chain functionalities, such as arginines. Recently, we demonstrated a novel approach that combines solid-phase synthesis and bioconjugation to obtain these derivatives with high efficiency and purity.^[3] The key step is chemoselective native chemical ligation (NCL) of 3'-cysteinyl-RNA fragments to highly soluble peptide thioesters. Based on our previously elaborated synthetic approach to generate 3'-aminoacylamino-3'-deoxyadenosine derivatives,^[4] we synthesized the novel cysteine functionalized adenosine solid support for standard solid-phase oligonucleotide synthesis. For the synthesis of the required peptide thioester fragments we took pattern on amino-modified benzylthioesters with an increased solubility in water.^[5] To extend the scope of this approach, we then used chemoselective metal-free desulfurization of cysteine-ligation products to form alanine-containing 3'-peptidyl-RNA conjugates.^[6] The so prepared 3'-peptidyl-RNA conjugates relate to resistance peptides that can render the ribosome resistant to macrolide antibiotics by a yet unknown ribosomal translation mechanism. By employing ribosome chemical probing experiments, we furthermore demonstrate that these 3'-peptidyl-RNA conjugates bind to their expected binding site in the PTC of the ribosome.

This native chemical ligation-desulfurization approach creates efficient access to hydrolysis-resistant, biologically active 3'-peptidyl-tRNA mimics. By involving NCL, higher side chain flexibility is achieved compared to the previously introduced route that relied exclusively on solid-phase synthesis.^[4] Using NCL we have obtained five resistance peptide-RNA conjugates^[3] that are awaited for structural and functional ribosomal studies to shed light on that specific antibiotic resistance phenomenon. Subsequent desulfurization of a cysteine-ligation product led to an alanine-containing 3'-peptidyl-RNA conjugate whose peptide moiety confers resistance to the macrolide antibiotic cethromycin.^[6]

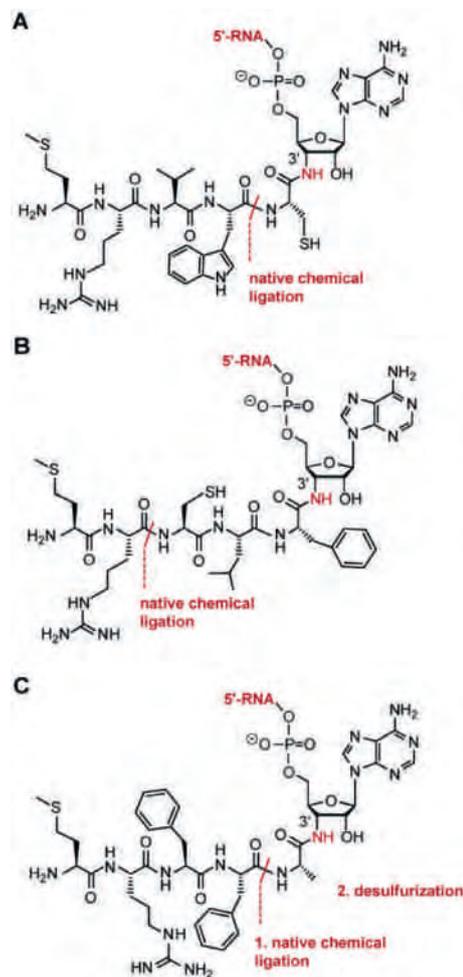


Figure 1. Novel strategy involving native chemical ligation and subsequent desulfurization for the synthesis of hydrolysis-resistant 3'-peptidyl-tRNA mimics. The shown peptide moieties represent sequences that confer resistance to the macrolide antibiotics telithromycin (A), josamycin (B), and cethromycin (C).

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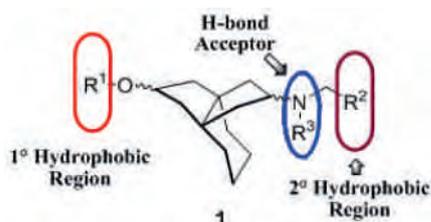
Design, Synthesis and Pharmacological Evaluation of Conformationally Restricted Selective Sigma-2 Receptor Ligands Based on the Propellane Scaffold

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Sigma (σ) receptors are widely distributed in the body and are classified in σ_1 and σ_2 subtypes. Whereas the σ_1 receptor has been cloned and pharmacologically characterized, the σ_2 subtype remains to be cloned.^[1] However, very recently the putative binding site of σ_2 receptors was identified.^[2] The σ_2 receptor has been linked with several pathological conditions such as psychiatric disorders, cocaine abuse, memory and learning disorders, dyskinesia and dystonic reactions induced by classical antipsychotic drugs, neuropathic pain, cholesterol synthesis, cancer and has been validated as a biomarker for tumor cell proliferation.^[3–3]

Due to the great therapeutic potential of σ_2 receptor ligands and as a part of our basic research about structural requirements for σ_2 selective ligands, in this work, novel σ_2 ligands were designed, synthesized and pharmacologically evaluated. For this purpose the highly rigid [4.3.3]propellane scaffold was used for stabilizing the three-dimensional arrangement of the pharmacophoric moieties required for high σ_2 affinity. The structure of the designed compounds **1** contain the three pharmacophoric elements that have been postulated to lead to high σ_2 affinity (see figure).^[4] A first series of stereoisomeric compounds of type **1** is currently under pharmacological evaluation.



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P409

Towards the Conception of Novel Antiparasitic Compounds: In Silico Studies on NAD⁺-Dependent Deacetylases

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Trypanosoma cruzi and *Leishmania* spp. are protozoan pathogens responsible of Chagas disease and Leishmaniasis, respectively. Current therapies rely only on a very small number of drugs, most of them inadequate because of their severe host toxicity or due to drug-resistance mechanisms.^[1,2] In order to find efficient therapeutic alternatives, the identification of new biotargets is highly desired. SIR2 proteins, NAD⁺-dependant deacetylases belonging to the sirtuin family, are known to be essential for the life cycle of both parasites and, for this reason, widely used in drug design.^[3,4] Recent studies also highlighted the therapeutic potential of other NAD⁺-dependant deacetylases.^[5] In this work, the structures of such enzymes isolated from *T. cruzi*, *L. infantum* and *L. braziliensis* have been retrieved by homology modeling techniques. A restricted number of chemical scaffolds, potentially active on both parasites, have then been identified through a virtual screening approach and energies of binding estimated through MM-PBSA calculations. Such in silico results are now forwarded for biological evaluation.

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P410

Polyhydroxylated Bicyclic Isooureas are Potent Glucocerebrosidase Inhibitors and Nanomolar Enzyme Activity Enhancers in Gaucher Cells

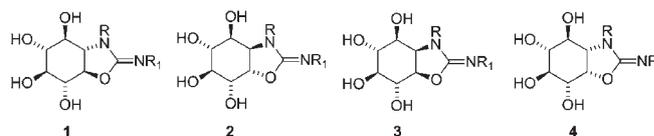
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Gaucher disease (GD) is the most prevalent lysosomal storage disorder caused by a deficiency in the activity of the enzyme acid β -glucosidase (GCase, β -glucocerebrosidase, EC 3.2.1.45).^[1] This leads to the accumulation of the substrate glucosylceramide in the lysosomes of macrophages causing severe symptoms. Current therapeutic strategies^[2] for GD involve either enzyme replacement therapy, or pharmacological GCase substrate reduction, which are of limited efficacy for disease variants affecting the central nervous system.

Recently, a third promising therapeutic option, the pharmacological chaperone therapy (PCT), has emerged. PCT is based on the use of reversible competitive GCase inhibitors that are capable of enhancing its residual hydrolytic activity at subinhibitory concentrations by stabilizing the functional form of the misfolded protein and preventing its premature degradation in the endoplasmic reticulum. This improves enzyme trafficking to the lysosome and enhances its hydrolytic activity. Thus, PCT is highly promising for GD, because it combines the benefits of the small-molecule approach, including oral bioavailability and the potential to cross the blood–brain barrier, with the specificity of an enzyme-directed approach.

In this context, four diastereomeric series of *N*-alkylated [6+5] bicyclic isooureas having hydroxyl substituents mimicking glucose hydroxyl groups have been synthesized as potential GCase inhibitors with the aim of developing pharmacological chaperones for GD.^[3] A strong effect of the stereochemistry of the cyclohexane nitrogen and hydroxyl substituents on the biological activity was evident in these families. When assayed on GCase, the isooureas displayed selective inhibition of GCase with low micromolar to nanomolar IC_{50} values in isolated enzyme experiments and also behaved as strong inhibitors of GCase in wild-type human fibroblasts. Among them, a family having a specific *cis* ring fusion (**4**) exhibited a strong inhibition against recombinant GCase with K_i values in the 2–42 nM range. The potential of these compounds as pharmaceutical chaperones was determined by testing their capacity of increasing GCase activity in GD lymphoblasts of the N370S and L444P variants, two of the most prevalent Gaucher mutations. Four compounds were selected from the different bicyclic isooureas obtained that increased GCase activity by 40–110% in N370S and 10–50% in L444P cells at low micromolar to nanomolar concentrations. These results describe a promising series of potent GCase ligands having the cellular properties required for pharmacological chaperones. The stereochemical influence of the substituents in other similar systems will be also reported.



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P411

A High-Quality Benchmark for Scoring Function Assessment

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Molecular docking methods rely on scoring functions to select ligand binding poses, to compute binding affinities, or to conduct other tasks. Various scoring functions have been developed in the past, and many more are still emerging. It is certainly desirable to assess the performance of these scoring functions on suitable benchmarks. The PDBbind database, which is currently maintained in our group, is a systematic collection of the experimental binding data of the protein–ligand complexes in the Protein Data Bank. It provides an ideal starting point for compiling such a benchmark. We have developed an approach for selecting the representing ones out of the protein–ligand complexes with high-resolution crystal structures and reliable binding data in the PDBbind database. Structural diversity at both the protein side and the ligand side is also emphasized during this process. The final outcome, namely the PDBbind core set (version 2011), consists of 216 protein–ligand complexes in 72 families. Based on this data set, a total of 20 popular scoring functions from both commercial software and academic groups were assessed in three aspects, i.e. “docking power”, “ranking power”, and “scoring power”. A number of general remarks regarding the performance of these scoring functions were derived for scoring function users and developers.

P412

Gaining Selectivity within the Gelatinase Subfamily: A Click Approach to Explore the Flexible S1' Pocket

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Matrix metalloproteinases (MMPs) are zinc endopeptidases that degrade the extracellular matrix (ECM) and are related to several pathological conditions.^[1] Among the MMP family, MMP-2 and MMP-9 constitute the gelatinase subfamily and share highly homologous catalytic domains. MMP-2 is a validated target for cancer therapy whereas MMP-9 is considered as an anti-target.^[2] Compounds that selectively inhibit MMP-2 over MMP-9 are thus sought for cancer therapy.

Our research group focuses on the selective inhibition of MMP-2, being particularly interested in finding hallmarks of selectivity over MMP-9, using a click-chemistry based approach. Recently we reported a family of clicked MMP-2 hydroxamic acid-type inhibitors (Figure 1).^[3] Those compounds have shown to be potent MMP-2 inhibitors with an interesting selectivity profile. However, they suffer from bad water solubility. A second family of similar clicked hydroxamic acids has been designed with the aim of improving its drug-like properties (Figure 1). Those compounds maintain the inhibition profile of the previously described family with nanomolar inhibition of MMP-2,^[4] and display higher solubility, reaching 10⁻¹ g/L values.

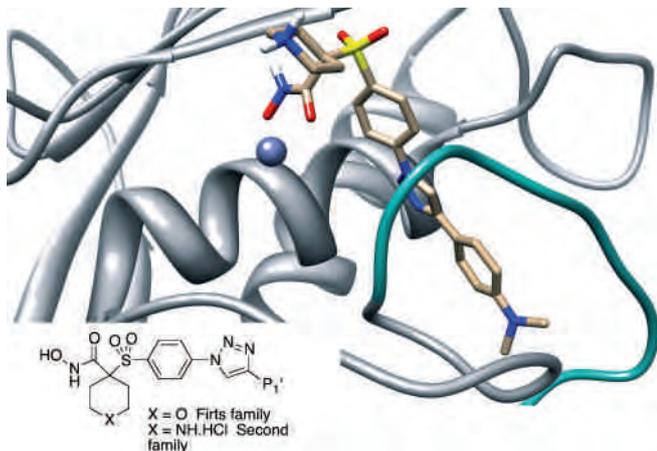


Figure 1. Snapshot extracted from a MD simulation (MMP-2). The flexible loop defining S1' pocket is depicted in blue. Inset: Chemical structure of the families of hydroxamic acids clicked to several P1' fragments.

The activities against MMP-2, MMP-9 and a kit of 10 MMPs were evaluated by colorimetric assays. Docking and NMR experiments (WaterLOGSY and STD competition) have been used to reveal the

potential interactions that govern the recognition and binding to MMP-2. 100-ns long molecular dynamics were carried out to rationalize the observed selectivity between the two gelatinases. These studies point out the difference of flexibility of the S1' pocket bottoms of gelatinases as the possible origin of the observed selectivity, and provide new insights into published experimental data. Anti-invasion and caco-2 assays are currently underway.

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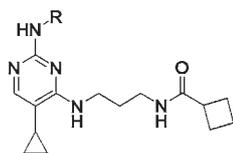
Synthesis and SAR of a Novel Series of 2,4-Diamino-5-cyclopropyl Pyrimidines as Selective Inhibitors of TBK1/IKKε

Edward G. McIver, Kristian Birchall, Thomas Drake, Stephen J. Lewis, Ela Smiljanic-Hurley, Joanne Osborne, William Tsang, Ahmad Kamal, Alison Levy, Michelle Raynor, Debra Taylor, Simon Arthur, Kristopher Clark, Philip Cohen

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Toll-like receptors (TLRs) play a key role in the activation of the innate immune system by invading pathogens (e.g., viruses, bacteria and fungi). Signalling via TLRs results in the activation of IκB kinases (IKKs), which regulate transcriptional programmes required for the production of inflammatory mediators to combat the invading pathogens. The canonical IKKs activate NF-κB leading to the production of pro-inflammatory cytokines, while the IKK-related kinases, known as TANK-binding kinase 1 (TBK1) and IκB kinase epsilon (IKKε) catalyse the activation of IRF3.^[1] Phosphorylation of IRF3 by TBK1/IKKε triggers its nuclear translocation and the subsequent expression of IRF3-dependent genes, such as interferon β. TBK1/IKKε also play an important role in restricting the extent of activation of the canonical IKKs.^[2,3]

We describe the development of a novel series of 2,4-diamino-5-cyclopropyl pyrimidines as potent inhibitors of TBK1, with good kinase selectivity and drug-like properties. These compounds have been evaluated in a range of cellular and in vivo assays, enabling us to probe the putative role of the TBK1/IKKε pathway in inflammatory diseases and cancer.^[4,5]



R	TBK1 IC ₅₀	IKK ϵ IC ₅₀
	6 nM	29 nM
	8 nM	31 nM

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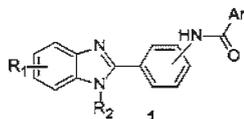
2-Arylbenzimidazole Derivatives as Anti-leishmanial Agents

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Leishmaniasis is listed as a neglected disease by the World Health Organization (WHO). It is a tropical disease transmitted by sandflies and caused by protozoan parasites belonging to the genus *Leishmania*.^[1] *L. donovani* and *L. infantum* are primarily responsible for visceral leishmaniasis that is fatal if untreated. Existing drugs suffer from poor compliance, toxicity, cost and parasite resistance. New treatments are urgently needed for this disease that affects millions of people mostly in developing countries.

Benzimidazole derivatives are known to possess a wide variety of biological activities, in particular antibacterial and antiviral activities, and as a privileged scaffold benzimidazole structure is a potential starting point to drug discovery. Now, we have found that 2-arylbenzimidazoles show promising activity against *Leishmania*. Design and synthesis of small library of 2-arylbenzimidazoles^[2] based on structure **1** are described, and the activity results of the compounds against *L. donovani* and structure–activity relationships are reviewed. Best compounds show good inhibition activity at low micromolar concentrations.



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P415

Pyridazine Derivatives Containing Azole Rings Behave as Fe-SOD Inhibitors and Show Remarkable Anti-*T. cruzi* Activity in Immunodeficient-Mouse Mode of Infection

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is currently a major health problem due to inadequate therapy and lack of an effective vaccine. The two drugs currently used to treat Chagas disease are the heterocyclic compounds nifurtimox and benznidazole, but both are scarcely effective in the chronic phase of the disease and result in significant side-effects, so that new molecules are urgently needed.^[1]

Following our research line on trypanosomicidal and leishmanicidal drugs,^[2] we have designed new 1-chloro-4-aminoalkyl- and 1,4-diaminoalkyl-substituted pyridazine derivatives containing pentaheterocyclic systems attached to the side chains. These compounds show remarkable activity against both the acute and chronic phase of the disease and also very low toxicity against human Vero cells. They are also able to strongly inhibit the antioxidant Fe-SOD enzyme of the parasite. Even more interestingly, assays performed on immunodepressed mice prevent reactivation of the infection and suggest a potential utility for treatment of patients with acquired immunodeficiency.^[3]

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P416

Bicyclic Isoxazol-3-one Analogues, a Novel Class of 5-HT_{2C} Receptor Agonists as Cognitive Enhancers

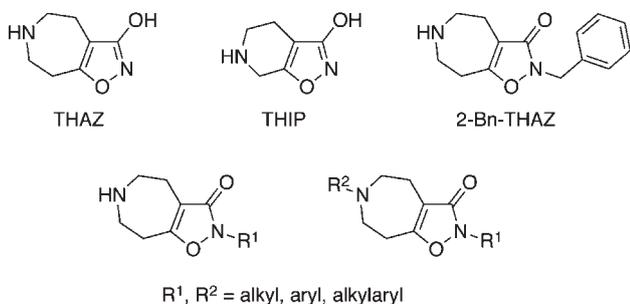
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The 5-HT_{2C} receptors appear to be expressed exclusively in the CNS, where they function as somatodendritic heteroreceptors on GABAergic, glutamatergic and/or dopaminergic neurons in several regions and are localized on GABAergic interneurons in the raphe nuclei. This modulatory role for 5-HT_{2C} receptors, for the activity in these three major neurotransmitter systems, has prompted the pursuit of the receptors as putative targets for a wide range of neurological and psychiatric disorders, epilepsy, sleep disorders, and obesity. A challenge in this respect has been to develop truly 5-HT_{2C}-selective ligands that do not concomitantly activate the highly homologous 5-HT_{2A} and 5-HT_{2B} subtypes.

5,6,7,8-Tetrahydro-4H-isoxazolo[4,5-d]azepin-3-ol (THAZ) is a seven-membered ring analogue of the functionally selective extrasynaptic GABA_A receptor agonist THIP/Gaboxadol. THAZ was originally designed for the GABA neurotransmitter system. Though, the scaffold of THAZ is structurally similar to a previously published 5-HT receptor agonist, *N,N*-diethyl-1-methylpiperidine-4-carboxamide, and to a fragment of a classical 5-HT receptor agonist, the hallucinogenic drug lysergic acid diethylamide (LSD).

A series of 2-substituted and 2,6-disubstituted THAZ analogues were synthesized and pharmacologically characterized at a plethora of monoamine receptors and other putative CNS targets. The therapeutic potential of the most promising analogue of the series has been investigated in animal tests for cognitive function and schizophrenia.



The 2-substituted THAZ analogues were shown to be moderately potent and relatively 5-HT_{2C} receptor selective. The most prominent of the analogues, 2-Bn-THAZ, showed substantially improved cognitive function in the mouse two-trial place recognition Y-maze assay, where the effect could be eliminated by co-administration of the 5-HT_{2C}-selective antagonist SB242084.

These results do not unequivocally link the 5-HT_{2C} component to its cognitive enhancement effect; they nevertheless suggest that this receptor could be an interesting target in disorders comprising cognitive dysfunction.

P417

Development of the First Orally Bioavailable PPARβ/δ-Selective Inverse Agonist

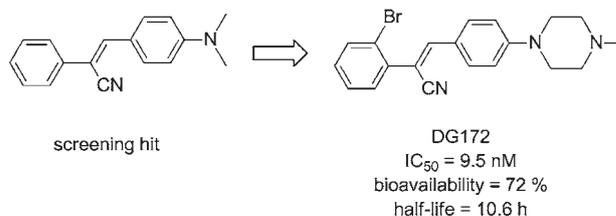
Frithjof Scheer,^[a] Sonja Lieber,^[b] Wolfgang Meissner,^[b] Simone Naruhn,^[b] Till Adhikary,^[b] Sabine Müller-Brüsselbach,^[b] Rolf Müller,^[b] Wiebke E. Diederich^[a]

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Peroxisome proliferator-activated receptors (PPARs) are a class of nuclear receptors consisting of three subtypes, namely PPARα, PPARβ/δ, and PPARγ. While drugs targeting PPARα and PPARγ concerning the treatment of one of the major health risk factors in western society, the metabolic syndrome, are already on the market, the role of PPARβ/δ still remains unclear. Unlike the other two subtypes, PPARβ/δ is expressed ubiquitously and modulates cell differentiation, proliferation, and inflammatory processes, but is also involved in fatty acid catabolism, energy metabolism, as well as reverse cholesterol transport. Endogenous ligands such as saturated fatty acids and eicosanoids act as agonists and thus enhance the coactivator recruitment of the active PPAR-retinoid X receptor heterodimer. Several synthetic partial and full agonists have already been described, but the number of ligands that reduce transcriptional activity of PPARβ/δ is very limited, although under certain pathophysiological conditions this reduction can have beneficial effects.^[1,2]

Due to the fact that none of the available PPARβ/δ antagonist is suitable for in vivo applications, we were seeking for a new chemical scaffold and thus screened 2693 compounds from the Open Chemical Repository of the NCI/NIH Developmental Therapeutics Program for PPARβ/δ inhibitory effects. One screening hit was used for a systematic SAR study revealing two modifications being crucial for affinity, the combination of which finally led to the design of DG172. This compound represents a noncovalent high-affinity binder for PPARβ/δ (IC₅₀=9.5 nM for C2C12 cells), enhancing the recruitment of corepressors and at the same time displaying a high-subtype selectivity. Most importantly, DG172 was found to be orally bioavailable in mice (72%) displaying a half-life of 10.6h within a biologically relevant concentration. Thus, DG172 has become a valuable tool to further elucidate the functions of PPARβ/δ in vivo.^[3]



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P418

Lead Identification Based on Bioaffinity FT-ICR-MS: Expanding the Scope of Structural Genomics

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Structural genomics consortia and structure-based drug discovery based on structures obtained by X-ray crystallography and NMR have been very successful. Even greater advances in cloning, expression and purification of proteins has underpinned this success. One outcome has been large numbers of proteins, in purified form, that have defied crystallization or solution structural studies. A technique that would allow identification of ligands binding to these proteins would facilitate lead identification.

Our approach has been based on natural products because of the similarity of recognition between biosynthetic enzymes and therapeutic targets.^[1,2] Protein fold topology (PFT) describes the imprint of biological interactions during biosynthesis and is a tool to interrogate protein structures of therapeutic interest.^[1,2]

We have observed ligand–protein complexes using FT-ICR-MS.^[3,4] We will report the validation of bioaffinity mass spectrometry on natural product fragments by demonstrating cellular and biochemical activity and our efforts to develop a heat map of the malaria proteome. These results offer expanded scope to structural genomics efforts.

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P419

Microsomal Biotransformation of a Series of Novel, Cytotoxic Benzenesulfonamide Derivatives. Metabolic Stability and Structure Elucidation of Potent Metabolites

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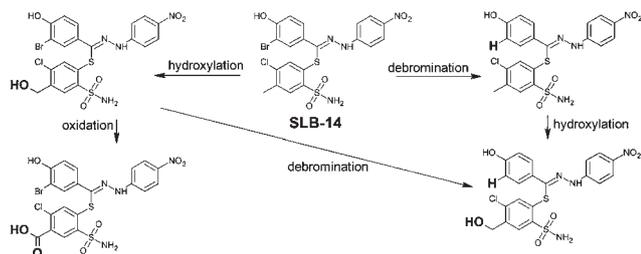
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A large number of drug candidates have undesirable properties, including low metabolic stability, which could result in poor bio-availability or weak efficacy. Metabolic stability is defined as the susceptibility of a chemical compound to biotransformation, and is expressed, for example as a half-life ($t_{1/2}$) determined in vitro using microsomal preparations obtained from human or other species livers. This experimental step should be included in drug development process, because it allows for determination of metabolic profiles of drug candidate early on pre-clinical stage, and, therefore, this information can be used to rationally plan the synthesis of new chemical entities with improved properties.

The synthesis of a new group of 4-chloro-2-mercapto-5-methyl-benzenesulfonamide derivatives with proven cytotoxicity against different cancer cell lines^[1,2] has started an in-depth biochemical project, describing their basic physicochemical properties (dissociation constant, solubility, lipophilicity), metabolic biotransformations, and leading to reveal mechanism of cytotoxic action. In addition, some chemometrics approaches were used, including principal component analysis, to demonstrate the relationship between chemical structure and activity within this group of compounds.^[3]

In this work, metabolic stability of selected derivatives was measured with particular emphasis on elucidation of metabolites structures using LC-MS and LC-MS/MS techniques. Human and rat liver microsomes were chosen as a model enzymatic systems to provide an approximation of phase I metabolism. Chromatographic analyses were performed at different time points till incubation end at 120 min. Poroshell EC120 C18 column (3 mm x 100 mm, 2.7 μ m) was used to obtain high selectivity and sufficient resolution for all of studied compounds. The scheme shown presents exemplary biotransformation pathway proposed for SLB-14 derivative, including debromination and methyl two-step oxidation to carboxylic acid. Similar results were obtained for other compounds. Further study will be aimed to define relation between susceptibility to formation of particular metabolite and derivative structure. Moreover, it is planned to point out CYP isoenzymes responsible for the above-mentioned reactions.



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P420

Polyfunctional Gene Transfection Agents

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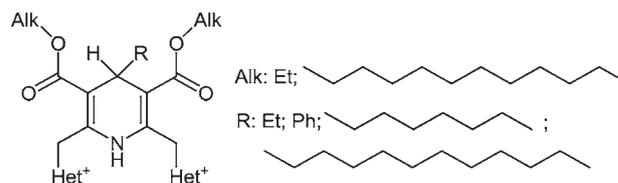
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Polypharmacology gains growing attention and is used as promising principle in medicinal chemistry for elaboration of novel drug candidates possessing several activities. The same approach could be used in case of gene or drug delivery agents. In several cases it has been mentioned that gene transfection agents possess also pharmacological activities.

We have studied and developed a new class of gene delivery agents: cationic amphiphilic derivatives of 1,4-dihydropyridine (DHP).^[1] This type of gene delivery agents possesses pharmacologically active scaffold of 1,4-DHP, which is declared as “privileged” due to multi-targeted and highly specific physiological activities (cardiovascular, neurotropic, antibacterial, anticancer, antimutagenic, etc.). Gene transfection of different eukariotic cell lines BHK-21, Cos-7, Huh-7, HepG2 was performed and compounds superior to popular commercial reagents have been checked. Free radical quenching activity was tested by making use of ABTS and DPPH (diphenylpicrylhydrazyl) methods.^[2]

Studies revealed high antiradical activity (ARA) of 1,4-DHP derivatives comprising electron-withdrawing pyridinio moieties in 2- and 6-methyl groups, so novel class of antiradical compounds is discovered including self-assembling DNA and RNA transfection agents. Steric factors for ARA are important: diminishing of ARA by long alkyl

chains in 3,5-ester groups was stated. Insertion of 4-alkyl substituents instead of 4-aryl groups increased gene transfection and RNA transport activities. By means of substituents in 2,6-pyridinimethylene moieties tuning of ARA is possible. Reversal of multidrug resistance by the amphiphilic 1,4-DHP derivative is an additional activity.^[3]



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P421

New Highly Selective FAK Inhibitors

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Focal adhesion kinase (FAK), a 125-kDa non-receptor tyrosine kinase, is primarily involved in controlling survival, proliferation, and motility of cells, with a potential role in oncogenesis. FAK overexpression has been observed in various cancer types including colon and breast. Therefore, FAK represents an interesting target for the development of kinase inhibitors. Among the published small-molecule FAK inhibitors, the bidentate diamino-pyrimidine PF-562271 was in clinical development and reported to be very selective, due to a direct interaction with a rare helical DFG-loop conformation.

In this paper, we describe for the first time a selectively substituted single-dentate hinge-binding pyridine scaffold (Figure 1). Beside the straight forward synthetic access to highly decorated molecules, SAR and properties will be discussed. We have been surprised to discover in structural analyses that some derivatives are more selective than aforementioned PF-562271, even without DFG interaction.

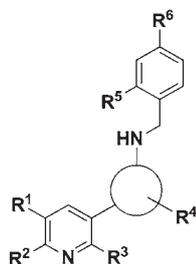


Figure 1. Pyridine scaffold as template for highly selective FAK inhibitors.

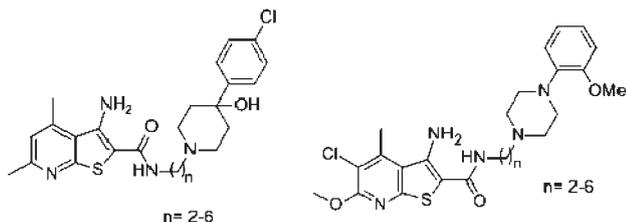
P422

Designed Multiple Ligands Targeting Dopamine D₂ Receptors and Muscarinic M₄ Receptors

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Schizophrenia is a debilitating and chronic disease state requiring ongoing treatment. Current antipsychotics target the positive and negative symptoms associated with this disease, predominantly via antagonism of dopamine D₂ receptors.^[1] However, there is an unmet need to address the cognitive dysfunction that also arises in schizophrenic patients. In this regard, positive allosteric modulation of the M₄ muscarinic acetylcholine receptor (mAChR) has emerged as a promising novel strategy.^[2] In the current study, we have synthesised a new set of designed multiple ligands (DMLs)^[3,4] as potential antipsychotics, that aim to concomitantly target dopamine D₂ receptors (as antagonists) and M₄ mAChRs (as positive allosteric modulators). We prepared two sets of DMLs (shown) that incorporate D₂ and M₄-targeting pharmacophores connected via varying linker lengths (2–6 carbons). Pharmacological evaluation of the activity of these analogues was determined using a cell-based ERK1/2 phosphorylation assay, in CHO cells expressing D₂ or M₄ receptors. Preliminary results around the SAR reveal that, attachment points of linkers and hybridizing the pharmacophore into smaller fragments at the M₄ receptor may be vital in maintaining allosteric activity. Similarly, we are able to maintain antagonism at the D₂ receptor.



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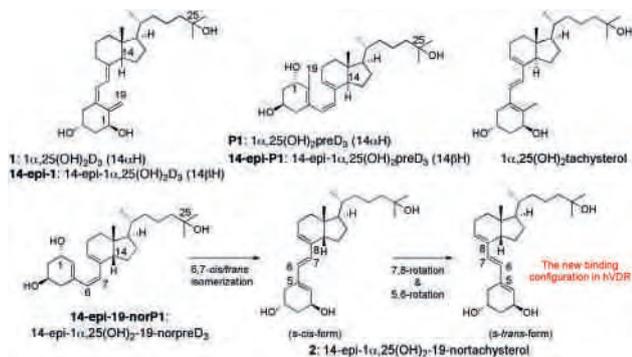
14-*epi*-19-Nortachysterol as a New Lead Compound in Vitamin D Family and Its Unprecedented Binding Configuration for the Human Vitamin D Receptor

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1 α ,25-Dihydroxyvitamin D₃ (**1**) is the most biologically active metabolite of vitamin D₃ and a ligand of the specific nuclear receptor (vitamin D receptor, VDR), although it contains 6% of its previtamin D form, 1 α ,25(OH)₂ preD₃ (**P1**), which is generated from **1** in thermal equilibrium through [1,7]-hydrogen shift. Regarding this equilibrium, 14-*epi*-vitamin D₃ shows a unique characteristic, that is, the pre-form of 14-*epi*-previtamin D₃ is major and dominant over 14-*epi*-vitamin D₃ in equilibrium. Recently, we have focused on the biological activity of the previtamin D form by synthesizing 14-*epi*-1 α ,25(OH)₂ previtamin D₃ (**14-epi-P1**) and also its 19-nor analogs, 14-*epi*-1 α ,25(OH)₂-19-norprevitamin D₃ (**14-epi-19-norP1**), which were able to lead neither to the [1,7]-hydrogen shift nor thermal equilibrium as natural vitamin D₃. In the study of the synthesis of **14-epi-19-norP1**, we found 14-*epi*-19-nortachysterol derivatives (**2**) through C6,7-*cis/trans* isomerization. This time, we are interested in these new tachysterol analogs, succeeded in their chemical synthesis and revealed their marked stability in comparison with natural tachysterol, which is easily converted to vitamin D₃ by UV irradiation and oxidized by O₂, and **2** showed their potent VDR binding affinity. Also, X-ray co-crystallographic analyses were performed using the complex of **2** with the ligand binding domain of the human VDR, and surprisingly, **2** exhibited an unprecedented binding configurations, C5,6-*s-trans* and C7,8-*s-trans* triene configurations, which were opposite the natural C7,8-*ene*-configuration of **1**. The superimposed binding configuration between **1** and **2** showed the suitability of the linker between A-ring and CD-ring, and flexibility of the CD-ring structure, and also the appropriate distribution of both the A-ring and the side chain in the ligand binding pocket was critical for high VDR binding affinity.



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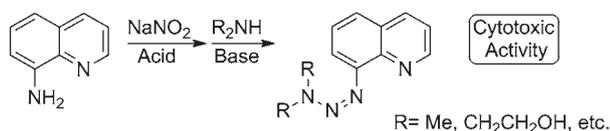
P424

Cytotoxicity of 1-Aryl-3,3-dialkyltriazenes which are Expected to Possess Chelating Ability

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1-Aryl-3,3-dialkyltriazenes have been used in many ways in organic syntheses and are known as anticancer drugs represented by dacarbazine. And we recently reported the triazene reacted rapidly to the ferric ion in the dilute condition (56 ppt). The triazene possessed *N,N*-bishydroxyethyl group on nitrogen 3 of triazenyl group. By the way, Desferrioxamine, PIH and Tachpyr possessed cytotoxicities and chelate to ferric ion recently. We tried to design and synthesis the triazenes which might possess chelating ability to ferric ion, and assessed about cytotoxicity of them. We synthesized some triazenes from some aminoquinolines and investigated the cytotoxicities of them toward human colon cancer LS-LM4 cell strain. Consequently, 8-aminoquinoline derivatives have strong cytotoxicities, especially, in the case of bishydroxyethyl group on 3-nitrogen of triazenyl group the most powerful cytotoxicities. And we suggested cell death was brought by apoptosis. To verify chelating ability of triazene with ferric ion, UV-Vis spectra of them were measured. Consequently, we suggested the cytotoxicity was concerned with Fe^{III} ion because the spectra were change addition of the 8-triazenyl quinolines.



P425

Exploring the Uncharted Pharmacological Space by Trifluoromethylated Cyclic Aliphatic Amines

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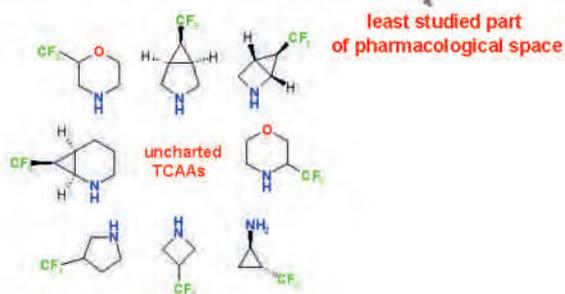
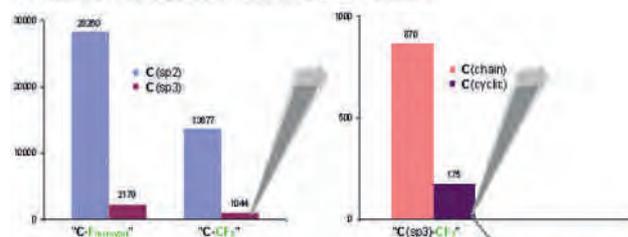
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Modern drug discovery is hard to imagine without fluorine, as ~20% of all pharmaceuticals contain this element. To date, however, only small part of the theoretically possible pharmacological space is covered. Many simple combinations of fluorine with other elements are still unknown, constraining thereby the probability to find new drugs that could benefit from a well-placed fluorine group.

Reduced molecular flexibility and enhanced number of sp³-carbons have been suggested as distinguishing features of approved drugs. Implementation of these principles leads to conceptually attractive space for drug discovery: fluorinated cyclic aliphatic motif. Analysis of MDL DDR database revealed that the trifluoromethylated part was the least populated within the known bioactives.

In this work, the chemical space covered by trifluoromethylated cyclic aliphatic amines (TCAAs) was explored. The possible origin for the rare occurrence of TCA motif in drug discovery was the following: the known TCAAs constituted only tiny fraction among all theoretically possible structures. Therefore, twenty novel TCAAs were selected and practical approaches to their preparation were developed.^[1-4] Enormous interest to these products from top pharmaceutical companies was observed once the compounds became commercially available.

Analysis of fluorinated compounds in MDL DDR database (March 2012)



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P427

Synthesis of Some New 3-Methyl-1-[(4-substituted-piperazin-1-yl)methyl]-1H-indole Derivatives and Their Cytotoxic Activity

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Cancer treatment has been a major attempt of research in academia and pharmaceutical industry for many years as it is one of the leading causes of death. Recent drug discovery efforts are highly focused towards design and synthesis of small molecules as anticancer agents due to the advantages of easier synthesis and lower cost.^[1–3]

A series of novel 3-methyl-1-[(4-substituted-piperazin-1-yl)methyl]-1H-indoles (**3a–l**) were synthesized and their cytotoxicities were analyzed against three different human cell lines, including liver (HUH7), breast (MCF7) and colon (HCT116). The Mannich reaction of 3-methylindole (**1**) with 4-substituted piperazines (**2**) and formaldehyde resulted to the 3-methyl-1-[(4-substituted piperazin-1-yl)methyl]-1H-indoles (**3a–l**) in 38–69% yields. Structure identification of the compounds was done by IR, ¹H NMR, ¹³C NMR spectra and elemental analyses. The cytotoxic activity of the synthesized compounds **3a–l** were screened on liver (HUH7), breast (MCF7) and colon (HCT116) cancer cell lines, by means of sulphorhodamine B (SRB) assays. Camptothecin was the positive control and 5-fluorouracil (5-FU) was used as the standard drug for the cytotoxic effect.

The investigation of anticancer screening revealed that the tested compounds showed comparable activity to 5-fluorouracil, and compounds **3g**, **3h**, **3i** and **3k** had lower 50% inhibition concentration (IC₅₀) than the reference drug in liver cell line, HUH7. The cytotoxic effects were not impressive against MCF7 breast cancer cells; all of the compounds showed cell viability with IC₅₀ values ranging from 13.69–68.81 μM. It was noteworthy that the cytotoxic effects were more pronounced against colon carcinoma cell line, HCT116. Similar to HUH7 cell line, compounds **3h** (IC₅₀=8.75 μM), **3i** (IC₅₀=15.91 μM) and **3k** (IC₅₀=16.62 μM) have better IC₅₀ values than 5-FLU (IC₅₀=18.78 μM) and also compound **3h** exhibited an IC₅₀ value of 8.75 μM, which represents good druggable cytotoxic activity. Moreover, the cytotoxic effect of the most potent compound **3h** on HUH7 and MCF7 cells through apoptosis was visualized by Hoechst staining and compared with paclitaxel, which is a mitotic inhibitor

acting on microtubules. The morphological features of apoptosis, i.e. condensation of chromatin and fragmentation of the nucleus, were examined. DMSO-treated control cells showed round and homogeneous nuclei, whereas **3h** and paclitaxel-treated cells showed condensed and fragmented nuclei.

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P428

Design of Novel and Selective SIRT6 Inhibitors as New Potential Therapeutic Agents in Cancer

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Sirtuins are a family of NAD-dependent enzymes that was proposed to control organismal life span about a decade ago. While such role is now being questioned, mounting evidences highlight the role of these enzymes in numerous physiological and pathological conditions, including metabolism, nutritional behavior, circadian rhythm, as well as inflammation and cancer. Studies show that SIRT6 is crucial for telomere maintenance, DNA repair and genome stability.^[1,2] Thus, SIRT6 inhibitors could conceivably be used to sensitize cancer cells to chemotherapeutics.

Here we present the design of novel drug-like and selective inhibitors of SIRT6 by means of structure-based drug design techniques. We used the X-ray structures of SIRT6 available in the Protein Data Bank and screened a large database of commercial compounds^[3] in a high-throughput docking campaign. The best scoring compounds were visually inspected and the final candidate selection was done taking in account the interactions of each molecule in the active sites and structural diversity of the molecules. A theoretical model aimed to predict Sirtuin selectivity was also obtained by using modeling techniques and the compound selection.

A total of 30 molecules were purchased and tested for their ability to inhibit SIRT6 using both fluorescence-based and immunoblots assays. Nine compounds were identified as active inhibitors of SIRT6 in both tests and four molecules showed IC₅₀ values at low micromolar range concentration. A selectivity profile of active compounds was carried out revealing that several of them have a 2.5 to 10-fold activity ratio on SIRT6 versus SIRT1 and SIRT2.

The structure-based approach used in this study lead us to the discovery of a series of new active and selective inhibitors of SIRT6 with different structural motifs from known sirtuins inhibitors. Synthetic efforts are currently pursued in order to obtain libraries to further explore their SAR and improve potency, selectivity and pharmacokinetics properties. These families of compounds represent interesting agents as potential novel anticancer therapies.

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P429

Heteroarylidene-3,5-dihydroimidazolones and 4H-Oxazolones as Calpain Inhibitors

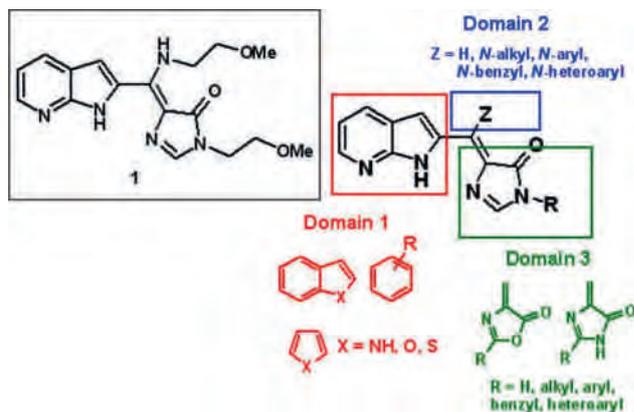
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The calpain family is a group of cysteine proteases unique in their dependency on calcium to attain functionally active forms. Calpains are involved in a wide range of cellular calcium-regulated functions, including signal transduction, cell proliferation and differentiation, and apoptosis. Moreover, altered calpain activity has been observed in several human diseases such as type 2 diabetes and metabolic syndrome.^[1] Therefore, calpains are an interesting target, and the design and synthesis of calpain inhibitors is an important area of research directed at the development of new potential pharmaceuticals.

Most of the synthetic calpain inhibitors reported^[2] are based on a peptidic structure, but in the last years promising non-peptide examples have also been identified. Our interest in the development of compounds targeting calpains involved in metabolic disorders lead us to discovery of the azaindole derivative **1** as a new and potent non-peptidic calpain inhibitor.

In this communication, we will report a structure–activity relationship (SAR) study on hit **1**, which is allowing us to identify some more potent analogues^[3] by introducing structural diversity on domains detailed in the figure.



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Development of Orally Available FimH Antagonists for the Treatment of Urinary Tract Infections

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Urinary tract infections (UTIs), including cystitis and pyelonephritis, affect a large proportion of the population and account for significant medical costs. In more than 80% of UTIs, uropathogenic *Escherichia coli* (UPEC) is the causative pathogen. The initial step in the pathogenesis of the infection is the adherence of UPEC to the human bladder epithelium, enabling the invasion into the host cells and the development of UTIs. This process is mediated by the lectin FimH located on type I pili enabling UPECs to attach to oligomannosides of the glycoprotein uroplakin Ia (UPIa) presented on uroepithelial cells. FimH antagonists such as α-D-mannopyranosides have been shown to interfere with the attachment of UPEC to their host cells, thus providing a novel therapeutic opportunity for the treatment and prevention of UTIs.^[1]

However, the pharmacokinetic properties of the tested glycomimetics do not meet the basic requirements of an oral treatment, because they exhibit only insufficient permeability through biological membranes. Since FimH antagonists contain a carboxylic acid moiety, a prodrug approach was envisaged. The esters of a series of FimH antagonists showed moderate water solubility but the expected high absorption potential by passive permeation.^[2] However, active efflux by P-glycoprotein caused an additional problem to be addressed by

structural modifications of the FimH antagonists. Moreover, the pro-drug approach is only applicable when the esters are readily cleaved by carboxylesterase. The rate of enzymatic hydrolysis of methyl esters of various FimH antagonists showed a strong structural dependence. FimH antagonists with a linear shape were more rapidly hydrolysed than those with a bent structure. Advanced kinetic studies suggest a higher affinity of the linear structures to the carboxylesterase and confirmed the different rates. With prodrugs of a series of new FimH antagonists, oral availability should be further improved.

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P431

In Vivo Activity and Mechanism of Action of a New 5-(Thiophen-2-yl)-Substituted 2-Aminobenzamide-Series Histone Deacetylase Inhibitor K-560

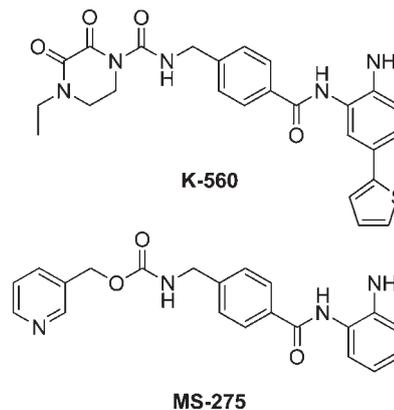
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We prepared a new orally bioavailable 5-(thiophen-2-yl)-substituted 2-aminobenzamide-type HDAC inhibitor **K-560** which had a 4-ethyl-2,3-dioxopiperazine-1-carboxamide group as a surface recognition domain.^[1] This compound showed promising HDAC1/2 inhibitory activities (HDAC1 IC₅₀: 0.05 μM; HDAC2 IC₅₀: 0.67 μM) as well as aqueous solubility (0.6 mg mL⁻¹ in 10% DMSO).

K-560 exhibited slightly stronger inhibition than **MS-275** against the growth of HCT116 cells and had a pharmacokinetic profile (C_{max}: 8.3 μM; t_{1/2}: 2.2 h) similar to HDAC inhibitors under development.^[1] Notably, unlike **MS-275**, this compound did not induce apoptosis even in 48 h in the cell cycle tests. This result suggested that **K-560** might have a more cytostatic effect on HCT116 cells than **MS-275** (2-thienyl-unsubstituted derivative). We thus conducted antitumor tests of **K-560** as well as **MS-275** as a positive reference compound, utilizing xenografts of HCT116 cells in nude mice. **K-560** suppressed the growth of tumor xenografts to T/C: 60% at 45 mg kg⁻¹ and 47% at 80 mg kg⁻¹. These values were close to the rate (T/C: 51% at 45 mg kg⁻¹) for **MS-275**. Furthermore, a loss of weight was induced by **MS-275** at 45 mg kg⁻¹, though not by **K-560** at either 45 mg kg⁻¹ or 80 mg kg⁻¹. This in vivo efficacy could be attributed to the cytostatic inhibitory effect of **K-560** on the growth of HCT116 cells, as suggested by the cell cycle tests. We thus subsequently focused on elucidation of mechanisms underlying the cytostatic activity. Western blot analyses indicated that, unlike **MS-275**, **K-560** induced neither cleaved PARP

nor cleaved caspase-3 in 24 and 48 h. It should be noted that **K-560** raised the level of autophagy-related protein LC3B,^[2,3] whereas **MS-275** did not. These results suggest the involvement of an autophagy pathway in suppression of the growth of HCT116 cells. Additional studies are underway to test this possibility.



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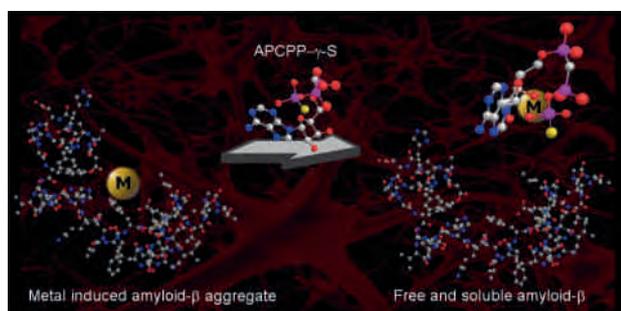
Nucleoside-5'-phosphorothioate Analogues are Biocompatible Antioxidants Dissolving Efficiently Amyloid Beta–Metal Ion Aggregates

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Amyloid beta (Aβ) peptide is known to precipitate and form aggregates with zinc and copper ions in vitro and in vivo in Alzheimer's disease (AD) patients. Metal-ion chelation was suggested as a therapy for metal-ion-induced Aβ aggregation, metal-ion overload, and oxidative stress. In a quest for biocompatible metal-ion chelators potentially useful for AD therapy, we tested a series of nucleoside 5'-phosphorothioate derivatives as re-solubilization agents of Cu⁺/Cu²⁺/Zn²⁺-induced Aβ aggregates and inhibitors of the Fenton reaction in Cu⁺ or Fe²⁺/H₂O₂ systems. The most promising chelator in this series was found to be APCPP-γ-S. This nucleotide was found to be more efficient than EDTA in re-solubilization of Aβ₄₀-Cu²⁺ aggregates as observed by the lower diameter (d_H: 86 versus 64 nm, respectively) obtained in dynamic light scattering measurements. Likewise, APCPP-γ-S dissolved Aβ₄₀-Cu⁺ and Aβ₄₂-Cu²⁺/Zn²⁺ aggregates, as monitored

by ^1H NMR and turbidity assays, respectively. Furthermore, addition of APCPP- γ -S to nine-day-old $\text{A}\beta_{40}\text{-Cu}^{2+}/\text{Zn}^{2+}$ aggregates resulted in size reduction as observed by transition electron microscopy (diameter reduction from 2.5 to 0.1 μm for $\text{A}\beta_{40}\text{-Cu}^{2+}$ aggregates). APCPP- γ -S proved to be more efficient than ascorbic acid and GSH in reducing OH radical production in $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ systems (IC_{50} =85, 216, and 92 μM , respectively). Therefore, we propose APCPP- γ -S as a potential AD therapy capable of both reducing OH radical production and re-solubilization of $\text{A}\beta_{40/42}\text{-M}^{n+}$ aggregates.



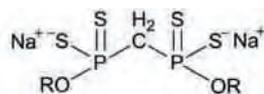
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Tetrathio Analogues of Bisphosphonate Salts as Metal Chelators and Antioxidants

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Soft/borderline metal ions such as Hg^{2+} , Pb^{2+} , Cd^{2+} , As^{3+} , and Cu^{2+} are known to be involved in several diseases and may cause poisoning. Moreover, high concentrations of redox-active metals can lead to oxidative stress in age-related diseases. Indeed, dimercaptosuccinic acid, unithiol, and dimercaprol are medically used metal chelators with a dithiol moiety. However, these metal chelators do not cross the blood–brain barrier. Moreover, dimercaprol is a toxic compound. Therefore, we synthesized several phosphorothioic compounds based on a bisphosphonate scaffold that is known for its medical use in chelating Ca^{2+} ions for the treatment of osteoporosis. The novel bisphosphonate scaffold forms a softer ligand that can chelate and detoxify soft/borderline metals. Furthermore, derivatives of phosphorodithioic acid are well known for their antiwear and antioxidant properties, which can help in reducing oxidative stress in various diseases. Methylene bis(dithiophosphonic-*O*-ester) analogues **1** were synthesized, and the antioxidant properties were evaluated in cell-free oxidative systems ($\text{Fe}^{2+}/\text{Cu}^{+}\text{-H}_2\text{O}_2$) by electron spin resonance (ESR). For these compounds, the IC_{50} values were around 100 μM for $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ and 55 μM for $\text{Cu}^{+}/\text{H}_2\text{O}_2$ systems in comparison to ascorbic acid (93 μM for $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ and >500 μM for $\text{Cu}^{+}/\text{H}_2\text{O}_2$ systems).



1
R=methyl, butyl, octyl, benzyl, propanenitrile

P434

Computational Model of the *E. coli* MurD Enzyme and In Silico Design of Novel Inhibitors of the Mur Ligase Family

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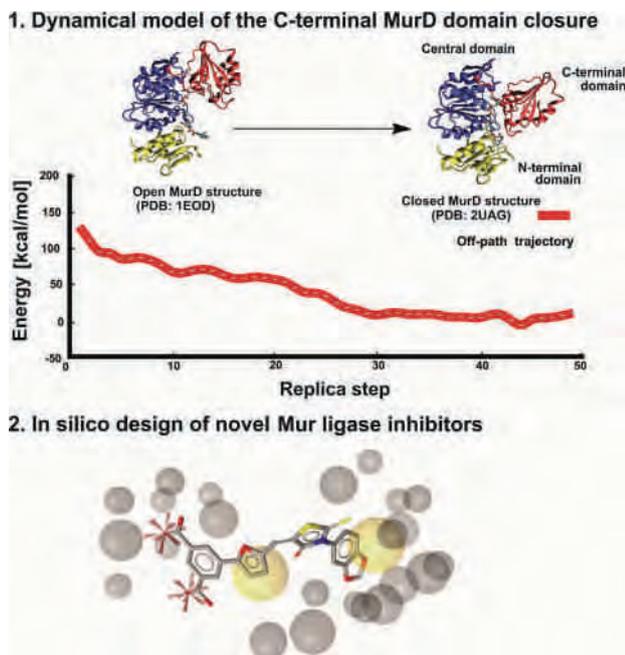
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The growing incidence of bacterial resistance to the available antibiotics renders the discovery of novel antibacterial agents imperative. An essential bacterial component, peptidoglycan, is traditionally an optimal target with respect to selective toxicity. In the peptidoglycan biosynthetic pathway, Mur ligases (MurC–MurF) catalyze the intracellular construction of its peptide moiety. MurD enzyme in particular catalyzes the incorporation of the D-Glu into the UMA precursor, coupled with concurrent ATP hydrolysis.

In our ongoing research, a complex dynamical model of the *E. coli* MurD enzyme was derived. To begin, targeted molecular dynamics (TMD) simulations were performed to examine the ligands' UMA and ATP binding processes and gain insight into the closure of the C-terminal domain.^[1] An off-path simulation (OPS) technique was initiated for the energy evaluation of the TMD-generated pathways.^[2] A QM/MM molecular modeling approach was utilized to evaluate reaction pathways leading to tetrahedral intermediate formation—a frequent drug design starting point.^[3] Calculated models confirmed the expected reaction order, first taking place between the ATP and UMA, resulting in the acyl-phosphate intermediate, followed by the addition of the D-Glu, which most likely enters the enzyme reaction in its deprotonated form. Binding free energies calculated for a series of MurD D-Glu-based inhibitors^[4] and their rigid surrogates revealed nonpolar van der Waals interactions as the main driving force for the binding of these inhibitors.

Based on the available structural data for the MurD and MurE enzymes, a virtual screening campaign was performed, resulting in the identification of a novel class of glutamic acid surrogates—benzene 1,3-dicarboxylic acid derivatives possessing dual MurD and MurE inhibitory activity.^[5] Subsequent design steps outlined 1,3-dicarboxylic acid derivatives with multiple Mur ligase (MurC–MurF) inhibition.



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P435

Mimetics of Peptides that Activate Kallikrein-Related Peptidase 3

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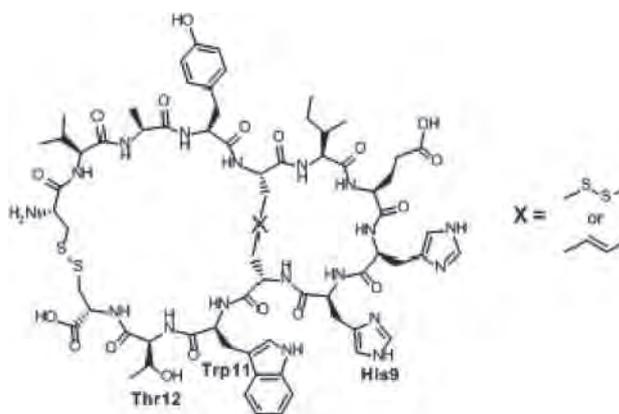
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Stimulation of the enzymatic activity of kallikrein-related peptidase 3 (KLK3, also known as prostate specific antigen, PSA) may be beneficial for patients with prostate cancer due to the antiangiogenic activity of KLK3.^[1,2] Only a few cyclic peptides have been reported

to stimulate KLK3; one of the most potent is peptide C-4 with 50% stimulation at a concentration of 0.57 μM .^[3] So far, no potent small molecules having the same activity have been discovered.

Our first efforts focused on replacing the non-terminal disulfide bridge in the peptide by hydrocarbon linkers. The important side chains of Tyr4, Ile6, His9, and Trp11 identified by an L-alanine replacement study, are located next to this disulfide bridge. Recently, concise routes to four orthogonally protected, enantiopure disulfide-bridge mimetics were reported by our group.^[4] Four reported mimetics had alkyne, (Z)-alkene, (E)-alkene, and alkane linkers as replacements for the disulfide bridge. The direct use of the building blocks in solid phase and solution phase peptide synthesis was more challenging than we had expected. The first pseudopeptides had to be synthesized with an alkene linker using a different strategy by performing a ring closing metathesis (RCM) reaction directly on the linear peptide having two L-allylglycine residues. The successful strategy included the synthesis of the peptide until the second L-allylglycine residue, then a RCM reaction on the uncompleted peptide, and finally synthesis of the rest of the peptide followed by cleavage of the final product from the resin. The RCM reaction on the full length peptide was unsuccessful. The pseudopeptide and the original C-4 peptide stimulated the activity of KLK3 2.2-fold and 4.5-fold, respectively, at a peptide concentration of 20 $\mu\text{g mL}^{-1}$. We also made some amino acid replacements in the peptide, which affect the biological activity of the pseudopeptide and the original peptide differently.

Further studies on the conformation of the original peptide and its proposed binding mode to a homology model of the enzyme suggested that the side chains of His9, Trp11, and Thr12 in the peptide are important. From that, we chose 4-quinolinone to be a suitable scaffold for the correct positioning of these important side chains. Our first 1,2,8-trisubstituted 4-quinolinone-based compounds were synthesized using a methodology with simple, commercially available starting materials to obtain 2-substituted 8-bromo-4-quinolinones, which were then further substituted via a Mitsunobu reaction and Pd-mediated coupling reaction at the 1- and 8-positions, respectively.



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P436

Nanoparticle: A Non-invasive Regulation Technique for Cellular Function

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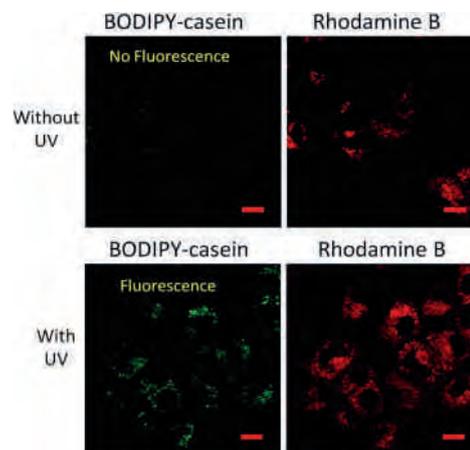
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We developed a novel method to regulate cellular function without invasion by using a photodegradable nanoparticle containing protein. Biological activities of the protein restricted by the nanoparticle encapsulation were recovered by applying external light. This technique enables us to directly control a variety of proteins activity and to regulate cellular function, whenever and wherever required in the cell, by UV irradiation.

Introduction: Almost all cellular functions are regulated by proteins. However, there are no universal and no invasive methods to control protein activities to regulate cellular functions whenever and wherever required in the cell. Therefore, a method of controlling protein activity in the cell to regulate cellular function is required. We have developed a nanoPARCEL method that can control protein activity in the cell using a photodegradable nanoparticle; however, the method requires microinjection to introduce the nanoparticle into the cell. To reduce the physical damage of microinjection, we improved the nanoPARCEL method, which induced the nanoparticle into the cell spontaneously.

Method: The cell surface is negatively charged; therefore, positively charged nanoparticles can enter the cell. We designed and synthesized a positively charged linker and made a positively charged photodegradable nanoparticle containing a protein or small molecule. The size of the nanoparticle was measured by dynamic light scattering and by transmission electron microscope. We observed cellular uptake and the stability of the nanoparticle within the cell by means of a fluorescence microscope.

Result: To demonstrate the internalization of the nanoparticle to the cell, a positively charged nanoparticle containing fluorescein was prepared. Within 10 min after addition of the nanoparticle to medium, over 90 % of cells showed green fluorescence, suggesting that the nanoparticle was taken into the cell by itself. To observe protein release from the nanoparticle, we prepared a nanoparticle containing BODIPY-casein. Green fluorescence was only observed in the cell that was UV irradiated. Moreover, it was shown that the nanoparticle remained after one week of incubation by using albumin-fluorescein as a marker.



We developed a positively charged photodegradable nanoparticle that can induce the cell without microinjection. In this method, there was no modification to the encapsulated protein individually, thus changing the encapsulated compound enabled us to regulate many cellular functions, whenever and wherever required.

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P437

Design, Synthesis and Biological Evaluation of Novel Benzoylurea Derivatives of Indolin-2-one Scaffold as Potent Aurora B Kinase Inhibitors

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Chih-Peng Liu,^[c] Yen-Chun Chen,^[c]
Chrong-Shiong Hwang,^[c] Ji-Wang Chern^[a]

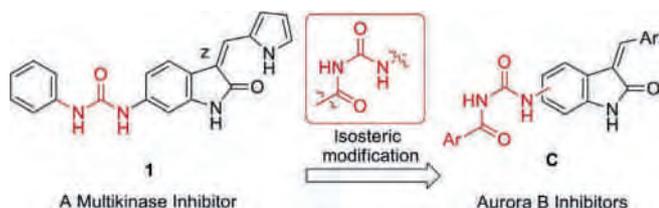
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Aurora kinases play a crucial role in cell division by the orchestration of pivotal mitotic events. Overexpression of these serine/threonine kinases have been noted in a variety of solid tumors (lung, liver, colon, breast, pancreatic tumors, etc.) and haematological malignancies such as acute myelogenous leukaemia. Perusal of literature reveals that the inhibition of Aurora kinases results in aberrant endoreduplication and apoptosis, illuminating their significance as an attractive target for the discovery of novel anticancer agents. As a part of our oncology portfolio, we have previously identified a urea derivative of indolin-2-one, **1**, as a multikinase inhibitor.^[1] In our ongoing research endeavor

to identify potent anticancer agents with an indolin-2-one core, a new class of compounds with a benzoylurea linker were rationally designed from lead molecule **1** by applying the concept of bioisosterism. Compounds from this new benzoylurea series have been found to inhibit Aurora B kinase selectively over other two paralogues, i.e. Aurora B and C, with single- to two-digit nanomolar IC_{50} values in enzymatic assay. Moreover, in vitro cell-based assays led to the identification of several lead compounds with IC_{50} values in the sub-micromolar to low micromolar range for their antiproliferative activity against the cell lines under investigation. Design, synthesis and structure–activity relationship (SAR) exploration of this series of compounds will be presented.



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P438

Adenosine A_{2A} Antagonists and Their Potential Use as Bivalent Ligands for the Treatment of Parkinson's Disease

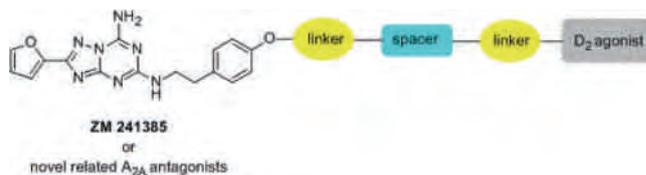
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Bivalent ligands are a relatively novel strategy towards targeting dimeric G protein-coupled receptor (GPCR) structures. Potential advantages such as enhanced potency and receptor subtype specificity, and improved pharmacokinetics compared to a multi-drug regimen, are the major driving forces behind this research. The design precept of bivalent ligands is paramount and aims to avoid any significant loss of biological activity or introduce other potential obstacles such as high molecular weight and increased lipophilicity.

Our research has been focused on the adenosine A_{2A} receptor, which is a relatively novel target for neuroprotection in Parkinson's disease. The literature compound ZM 241385 was used as a starting point from which a series of novel adenosine A_{2A} antagonists was synthesized and tested in a cAMP-related functional assay. Our research group extensively investigated the biological effects of different kinds of linkers attached to various positions on the adenosine A_{2A} antagonists to allow further chemical elaboration.

Our future work will focus on the synthesis and biological testing of heterobivalent ligands that target the A_{2A} – D_2 dimer as a novel treatment for Parkinson's disease. The designed heterobivalent ligands will incorporate ZM 241385 or other novel related adenosine A_{2A} antagonists linked to a dopamine D_2 agonist.^[1,2]



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P439

Influence of Polyphenol–Protein Interaction on the Antioxidant Activities of Polyphenols

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Background: Polyphenols in plasma are bound to plasma proteins to some degree. The polyphenol–protein interaction (PPI) is reversible in that the polyphenol–protein complex can dissociate and release the free polyphenols.^[1,2] Polyphenols and their metabolites rapidly exchange between free and bound forms within the circulation. The PPI is expected to modulate the bioavailability of polyphenols. It looks like the effect of the polyphenol–protein interaction on the bioavailability of flavonoids is not equivocal, and the interaction of flavonoids with proteins weakens the antioxidant capacity of the flavonoids.^[3–4] The reversible and irreversible PPIs depend on pH, temperature, and concentrations of protein and polyphenols.^[5] The biological effects of polyphenol–protein complexes on bioactivities of polyphenols are still not clear. Determining the influence of PPIs on the antioxidative ability of polyphenols is critical and will directly correlate with the bioavailability of polyphenols. Herein, the influence of PPIs on the DPPH free radical scavenging potential of polyphenols was investigated in detail. Forty-six polyphenols were studied.

Materials and Methods: The antioxidant activities of polyphenols (1.0×10^{-3} mol L^{-1}) in the absence and presence of BSA (1.0×10^{-5} mol L^{-1}) were measured on the basis of the DPPH radical scavenging activity. One milliliter sample was added to 1 mL of DPPH solution (0.2 mmol L^{-1} in ethanol) as the free radical source. The decrease in the solution absorbance was measured at 517 nm after 30 min.

Results and Discussion: The working solutions of the polyphenols (1.0×10^{-3} mol L $^{-1}$) were diluted ten times with water or BSA solution (1.0×10^{-5} mol L $^{-1}$) to obtain tested samples. The immediate DPPH radical scavenging activities of the tested samples were detected to illustrate the influence of PPI on the DPPH scavenging potential of polyphenols. The result showed that the PPI rapidly weakens the DPPH radical scavenging activity of polyphenols. The DPPH radical scavenging capacities of several polyphenols in the presence of BSA completely disappeared. As shown in Figure 1, the DPPH radical scavenging capacities of polyphenols are obviously reduced. Polyphenols with higher DPPH scavenging potential exhibited more obvious decreases in DPPH scavenging potential in the presence of BSA. The working solutions of polyphenols (1.0×10^{-3} mol L $^{-1}$) were diluted ten times with buffer or BSA solution (1.0×10^{-5} mol L $^{-1}$) and kept at 25°C for 7 days to obtain tested samples. The 7th-day DPPH radical scavenging activities of the tested samples were detected to illustrate the effect of the BSA–polyphenol interaction on the DPPH scavenging potential of polyphenols. Except for kaempferol, which showed the highest activity, BSA significantly enhances the DPPH scavenging activity of polyphenols after being kept in room temperature under aerobic conditions for 7 days. For kaempferol (1.0×10^{-4} mol L $^{-1}$), DPPH scavenging percentages in the absence and presence of BSA were 80.19% and 64.65%. The DPPH scavenging percentages of other polyphenols (1.0×10^{-4} mol L $^{-1}$) after incubation with BSA for 7 days were mostly distributed between 60% and 80%. The DPPH scavenging capacities of polyphenols without antioxidant potential in the presence of BSA were enormously improved. Polyphenols with higher DPPH scavenging potential showed smaller increases in DPPH scavenging potential in the presence of BSA. The lipophilicity of the compounds under study was assessed by their partition coefficient values (XLogP3) according to the PubChem Public Chemical Database. The DPPH scavenging percentages of polyphenol–BSA complexes decreased with increasing partition coefficient. The DPPH radical scavenging percentages (%) of polyphenol–BSA complexes were slightly increased with increasing hydrogen bond donor numbers of polyphenols.

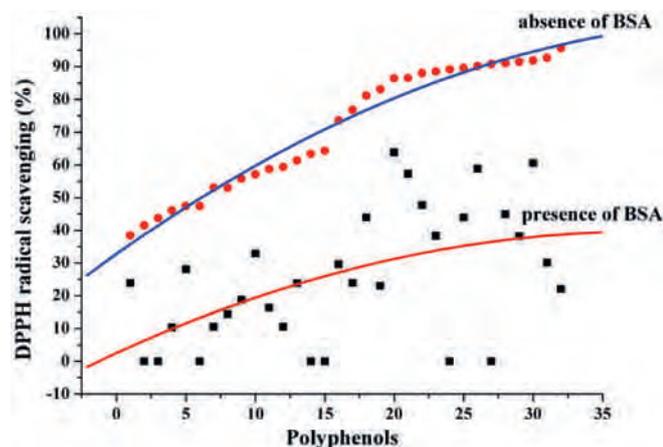


Figure 1. The immediate DPPH radical scavenging capacity of polyphenols in the presence and absence of BSA under aerobic condition.

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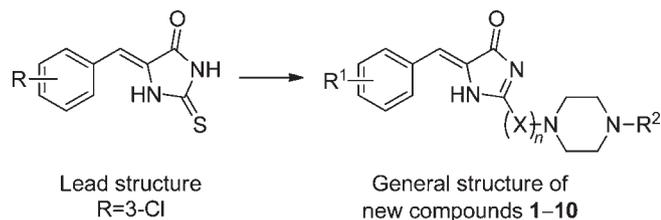
Search for Chemosensitizers of Bacterial MDR Efflux Pumps among Novel Piperazine Derivatives of 5-Arylideneimidazolone

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Multidrug resistance (MDR) is a serious therapeutic problem in case of bacterial,^[1] fungal,^[2] and cancer diseases.^[3] In each case, one of the main mechanisms of MDR involves protein efflux pumps, e.g. membrane transport proteins, which are able to extrude drugs and antibiotics before they can reach their targets. A main strategy to overcome MDR is focused on the search for new chemical compounds, so-called efflux pump inhibitors (EPIs), that are able to block MDR pump functions by competitive or noncompetitive inhibition as well as by wasting energy necessary for pump activation. The EPIs are useful for wider studies on MDR mechanisms on a molecular level and could improve therapy of antibiotics as “adjuvants” in the future. Our previous studies concentrated on (thio)hydantoin derivatives and indicated the moderate abilities of some compounds to increase antibiotic susceptibility to resistant *Enterobacter aerogenes* strains.^[4] Among others, 5-arylidene derivatives of thiohydantoin (see figure) decreased the minimal inhibitory concentration (MIC) of antibiotics, but their properties were significantly limited by very low solubility. Thus, the present studies are focused on new chemical modifications of an arylidenehydantoin lead structure (5-(4-chlorobenzylidene)-2-thiohydantoin) to give a series of 2-piperazinyl derivatives of arylideneimidazolones, differing within three structural fragments: (1) aromatic substituents at position 5; (2) length of linker between imidazolone and piperazine moieties; and (3) substituents at the piperazine-terminated fragment (see figure). Synthesis of the new compounds was performed within three to four steps using Knoevenagel condensations, S-methylation, N-alkylation, and/or N-deprotection processes. The novel compounds

were evaluated for their abilities to improve efficacy of antibiotics in three strains of *E. aerogenes*. SAR studies were performed. Active chemosensitizers were found among compounds with free piperazine-terminal fragments, whereas the presence of substituents at both piperazine nitrogen atoms significantly diminished the abilities of the compounds to improve antibiotic efficacy.



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P443

Exploring the Orthosteric nAChR Binding Site by Fragment Growing of the nAChR Agonist DMABC

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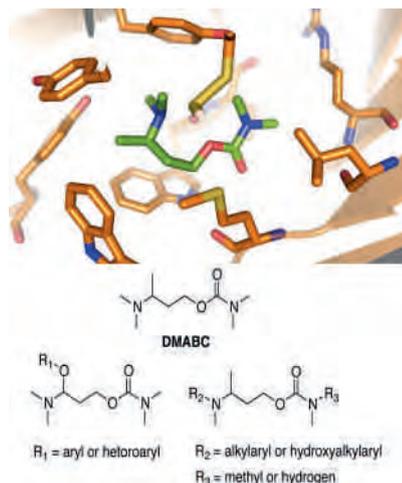
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The nicotinic acetylcholine receptors (nAChRs) are expressed throughout the central and peripheral nervous systems, where they play essential roles in numerous physiological processes. Consequently, therapeutic intervention in nAChR signalling has proven beneficial in many neurodegenerative and psychiatric disorders like Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia, pain, and depression. The nAChRs are one of the major receptor classes of the super family of ligand-gated ion channels (LGICs). The nAChRs are pentameric assemblies of subunits (α 1–10, β 1–4, γ , δ , and ϵ), which form an ion channel either as homomeric or heteromeric receptors.

In recent years, highly valuable insight into the molecular architecture of the LGIC receptor complex has been obtained because several high-resolution X-ray crystal structures of acetylcholine-binding proteins (AChBPs) from various snails and some bacterial ion channels have been solved. The AChBPs display significant amino acid sequence homologies with the ligand binding domain

of the LGICs and are thereby excellent templates for homology modelling of these domains, especially the ionotropic nAChRs and GABA_A receptors.

The compound 3-(dimethylamino)butyl dimethylcarbamate (DMABC) is an acetylcholine-related compound that exhibits high selectivity for neuronal over muscarinic nAChRs and significant selectivity towards the $\alpha_4\beta_2$ subtype over the $\alpha_3\beta_4$ and the α_7 subtypes.^[1] The design of previously synthesized DMABC analogues was based on docking into homology models of the nAChRs, but recently, an X-ray crystal structure of DMABC co-crystallised with AChBP was solved, giving new information about the binding mode of the ligand.^[2] New DMABC analogues were designed based on the X-ray crystal structure, and fragment growing was applied to give access to an additional identified cavity associated to the orthosteric binding pocket. Several analogues were chosen for synthesis based on docking scores. Pharmacological testing of the synthesized analogues in a [³H]epibatidine binding assay at the $\alpha_4\beta_2$, $\alpha_3\beta_2$, and $\alpha_4\beta_4$ subtypes and a FLIPR Membrane Potential Blue assay at the $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes revealed new important information about the binding mode of the DMABC analogues.



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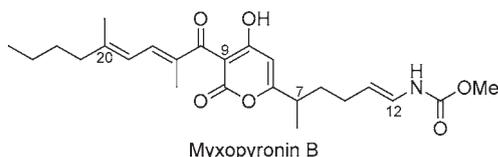
P445

Novel Hybrid-Type Derivatives of Myxopyronin Targeting Bacterial Infections

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The bacterial RNA polymerase (RNAP) is the essential organ for the survival of bacteria. Therefore, RNAP is the ideal target of antibiotics. In recent years, myxopyronin was isolated from *Myxococcus fulvus* Mxf50 and showed selective inhibitory activity against RNAP. In addition, myxopyronin was found to bind the new binding site in RNAP, which exists at the hinge part of RNAP and exhibits the opening and closing movement of a strand for transcription. The novel inhibitory mechanism of myxopyronin on the basis of its binding site was expected to lead to a new antibiotic for the treatment of resistant bacteria. However, high lipophilicity had to be improved for activity enhancement and a broad antibacterial spectrum. Hence, we tried to produce the hybridized compound of myxopyronin. As a functional molecule, holomycin was selected, which had low lipophilicity and a moderate but broad antibacterial spectrum. In the presentation, we report synthetic studies on novel hybrid-type derivatives of myxopyronin and holomycin. Synthesized derivatives were evaluated in vitro for antimicrobial activity and inhibitory activity against bacterial RNAP. In addition, the binding sites of our compounds were investigated using a molecular modeling study.



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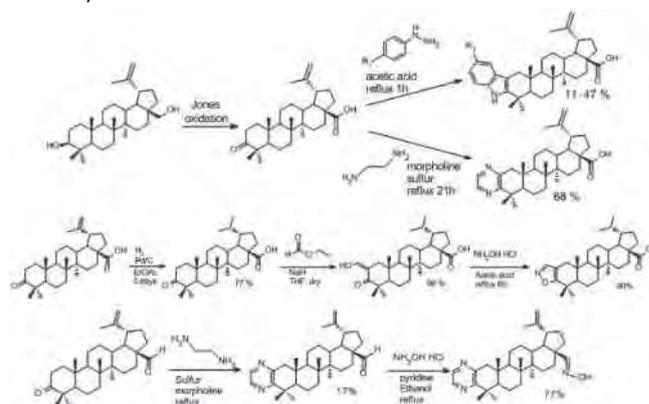
Heterocyclic Betulin Derivatives against Protozoan *Leishmania* Parasites

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The (chemo)therapy of leishmaniasis is a neglected area of research and drug development. Current treatments of leishmaniasis are unsatisfactory in terms of safety and efficacy. Our group has previously shown that heterocyclic betulin derivatives inhibit growth of *Leishmania donovani*.^[1] Betulin is the principle extractive substance of the outer birch (*Betula* sp.) bark, which is a low-value waste product of the forest industry.

Betulin was converted to betulonic acid, which is a versatile intermediate, by the Jones procedure. Indole derivatives of betulonic acid were synthesized by classic Fisher indole synthesis, and a pyrazine derivative was synthesized by sulfur catalysis in morpholine. The pyrazine derivative of betulonic aldehyde was synthesized and its formyl group was converted to an oxime group. An isoxazole derivative of betulonic acid was synthesized via α -hydroxymethylene ketone. The products have been tested against *L. donovani* at the Department of Microbiology and Molecular Genetics, IMRIC, the Hebrew University of Jerusalem (Israel). Of these compounds, the pyrazine derivative of betulonic acid was the most active one. When the carboxyl group was changed to an oxime group, while keeping the pyrazine ring, activity was lost. On the other hand, the isoxazole derivative of betulonic acid was inactive. Based on these results, synthesis will be continued towards more active and more soluble compounds. Other different kinds of fused heterocycles will be synthesized including pyridine, pyrazole, and thiophene derivatives. Also, the carboxyl group of the heterocyclic derivatives will be modified.



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P448

Discovery of Insulin-Degrading Enzyme Modulators: Challenges for the Validation of a Cryptidase as a Biological Target

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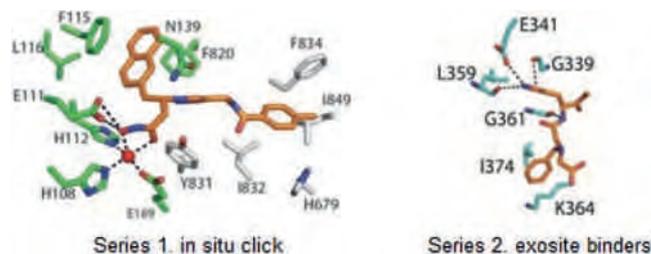
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Insulysin or insulin-degrading enzyme (IDE) is a ubiquitous zinc metalloprotease implicated in the clearance of numerous physiological peptides. Insulysin hydrolyses, in particular beta-amyloid peptide and insulin, are respectively implicated in Alzheimer's disease and diabetes. Also, it cleaves several peptides such as the insulin-like growth factor 2 (IGF-2), atrial natriuretic peptide (ANP), the transforming growth factor- α (TGF- α), amylin, glucagon, bradikinin, somatostatin, and others. Consistent with the fact that IDE degrades insulin in vitro, mutations leading to functional loss of IDE in mice result in high levels of insulin and development of glucose intolerance. Moreover, genetic studies have shown that polymorphism on the IDE region of chromosome are associated with type-2 diabetes and Alzheimer's disease.

The X-ray structure of human IDE by Tang et al. (*Nature* **2006**) has given molecular insight for the understanding of substrate recognition. The N- and C-domains of insulysin, linked by a 28-amino-acid loop form a large catalytic cavity ("crypt") that can accommodate peptides as long as 70 amino acids. Once trapped in this catalytic chamber, substrates undergo conformational changes that help their interaction with two key regions: in X-ray structures, substrates are bound to the enzyme, both to the catalytic site and to an exosite that sits at about 30 Å from the catalytic zinc atom.

We present here two series of modulators of this unique enzyme that will help in understanding its role in the physiological processes. The first series was discovered by an original orthogonal in situ click reaction, allowing the first drug like zinc-binding inhibitors of this enzyme. The second series of modulators was discovered by screening and further optimization. Several X-ray structures revealed an original binding mode, and cell-based assays provided data to support the in vitro enzymatic results. Our study thus provides the first drug-like small-molecule modulators of IDE that bind the exosite and modulate the enzyme differently for each substrate. This work opens up realistic ways to control the activity of an enzyme in a pathway-dependent manner. The mode of action of our compounds opens new avenues both for the study of the function of IDE and for the design of therapeutic interventions.



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Synthesis of Tetrabenazine via Intramolecular Aza-Prins-Type Cyclization

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Tetrabenazine (TBZ, xenazine), first introduced as an antipsychotic in the 1950s, was approved by the US FDA for treatment of chorea associated with Huntington's disease. Although various methods for the construction of this class of heterocycles are reported, the development of new efficient methods to synthesize such compounds are still challenging to the synthetic organic communities due to their structural novelty and application. The Prins cyclization reaction is known as one of the useful synthetic strategies to construct five- or six-membered ring systems containing oxygen or nitrogen heteroatoms. In this study, we report the total synthesis of the tetrabenazine alkaloids through an intramolecular aza-Prins-type cyclization of an amino allylsilane using an oxidative C–H activation. The synthetic approach toward tetrabenazine starting from the cyclized product will be discussed also.

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P450

PPAR β/δ Inhibitors Based on the Structure of GSK0660

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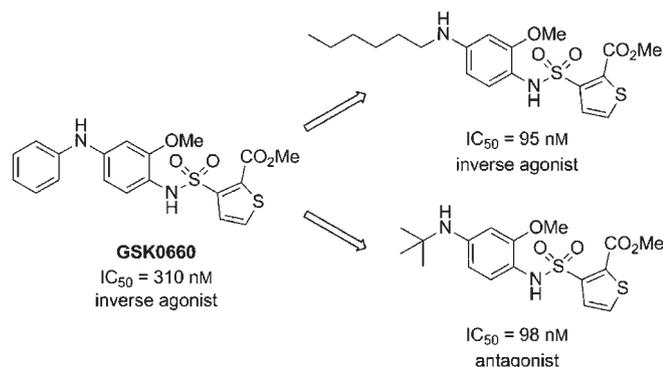
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The peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors involved in metabolism and inflammation, as well as the cell life-cycle. The three PPAR subtypes (PPAR α , PPAR β/δ and PPAR γ) form heterodimers with the retinoid X receptor and regulate their target genes through binding to specific DNA elements (PPREs) in combination with binding of either a coactivator or corepressor peptide depending on the nature of a respective ligand.

PPAR α is the target of the fibrate class of hypolipidemic drugs whereas PPAR γ is addressed by anti-type II diabetes thiazolidinedione drugs. For PPAR β/δ , most of the published ligands show agonistic properties. The few inhibitory ligands published until recently all showed either a lack of selectivity or bioavailability. To elucidate the molecular and biological functions of PPAR β/δ , the development of selective inhibitory ligands for PPAR β/δ is of utmost importance.

Based on the structure of GSK0660,^[1] the first published inhibitory ligand for PPAR β/δ , we synthesized a series of PPAR β/δ -selective derivatives which possess up to 10-fold improved binding affinities when compared to GSK0660.^[2] While most of these ligands feature inverse agonistic properties, some in fact proved to be pure antagonists lacking the ability to recruit corepressor peptides. Thus they were able to diminish both the ligand-induced binding of coactivators as well as corepressors.^[3]



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P451

Salphen Metal Complexes as Selective Quadruplex DNA Binders

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Guanine-rich sequences of DNA can assemble into quadruply-stranded structures known as quadruplexes.^[1] Many of these sequences are found in the promoter regions of genes and hence it has been proposed that they might play important roles in regulating gene expression, for example, of certain oncogenes such as c-myc and c-kit.^[2] This observation has opened the opportunity for designing molecules with potential anticancer activity whose action is based on their ability to stabilize quadruplex DNA structures associated with the regulation of oncogenes.^[3] Indeed, the number of molecules designed to stabilize the G4 structure is rapidly increasing. Among these molecules, metal complexes have emerged as excellent quadruplex DNA binders.^[4]

An encouraging sign of the potential druggability of this target is the fact that there is already one G4-binder undergoing phase II clinical trials.^[5] However, there is still an important challenge to solve in order to bring more families of G4-binders to the pharmaceutical industry: their selectivity for quadruplex over duplex DNA needs to be improved. Although some of the compounds reported to date have shown considerable selectivity, this is still not as high as it should ideally be.

The results that will be presented in this paper focus on improving the selectivity of salphen metal complexes, which were one of the first types of metal complexes to be shown by our group to be excellent quadruplex binders. While maintaining the excellent binding constants found for these complexes,^[6] the aim was to obtain metal complexes with no binding or relatively low binding to duplex DNA. We will present results indicating how, by rational modification of the substitution pattern around the metal salphen planar core, it is possible to achieve different affinities and improve selectivities. Parameters such as charge of the substituents, position and bulkiness have been studied. The results of our findings will be presented with particular attention to sulfonic-substituted salphen complexes, which showed the ability to modify the binding properties of the corresponding metal complex. In addition, we will present computational docking studies carried out to rationalize the binding mode of these complexes towards the G4 DNA.

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P452

Structure–Activity Study of New Analogues of Cyclo-prolyglycine

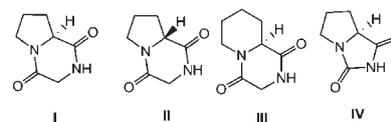
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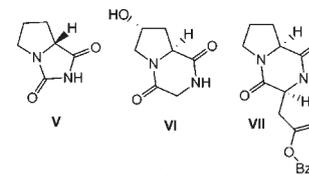
Cyclo-prolyglycine (CPG, I) was initially designed as peptide analogue of classical nootropic piracetam,^[1] and it was then found in the rat brain as an endogenous compound.^[2] CPG not only shared similar chemical structure with piracetam but produced similar spectrum psychotropic activities including nootropic, anxiolytic and antihypoxic activities.^[3,4] For revealing pharmacophores responsible for these activities of CPG, we synthesized its structural analogues: its enantiomer (II), a compound with an expanded pyrrolidine cycle (III), enantiomers of the compound with a narrowed piperazine cycle (IV, V), with substitution in the pyrrolidine cycle (VI), and with substitution in the piperazine cycle (VII).

Compounds I–III, VI and VII were retrieved by cyclization of dipeptides synthesized by classic methods of peptide synthesis in solution: by the activated esters method or by the mixed anhydrides method under Andersen conditions. Compounds IV and V were obtained by conjugation of proline with urea with heating in the presence of sulfuric acid. Nootropic properties were investigated in an experimental model of passive avoidance test in rats with electroconvulsive or scopolamine-induced amnesia. Anxiolytic activity was examined in the elevated plus-maze test in rats. Antihypoxic activity was studied in the experimental model of normobaric hypoxia with hypercapnia in mice.

It was shown that chirality change (I to II) results in inverse of nootropic and loss of antihypoxic and anxiolytic activities. Pyrrolidine cycle expansion (compound III) leads to nootropic activity abolishment and antihypoxic and anxiolytic activity conservation. Downsizing of the piperazine cycle (compound IV) induces abolishment of antihypoxic activity and conservation of nootropic and anxiolytic activities. Chirality changes (IV to V) results in loss of nootropic and antihypoxic activities and inversion of anxiolytic activity. Substitution in the pyrrolidine cycle (compound VI) leads to inversion of nootropic and antihypoxic activities and conservation of anxiolytic activity. Substitution in the piperazine cycle (compound VII) induces conservation of nootropic, antihypoxic and anxiolytic activities. These results indicate an absence of parallelism between structural changes in series of analogues of CPG and changes in anxiolytic, nootropic and antihypoxic activities. It can be suggested that pharmacological targets responsible for different types of psychopharmacologic activity do not coincide.



Activity:	I	II	III	IV
nootropic	(+)	(-)	(0)	(+)
anxiolytic	(+)	(0)	(+)	(+)
antihypoxic	(+)	(0)	(+)	(0)



Activity:	V	VI	VII
nootropic	(0)	(-)	(+)
anxiolytic	(-)	(+)	(+)
antihypoxic	(0)	(-)	(+)

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P453

Development of New 7-Morpholino-4-quinolone-3-carboxamide Derivatives as Potential New Drugs against *Trypanosoma brucei*

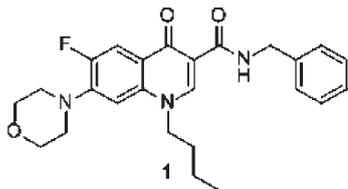
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Human African trypanosomiasis (HAT), or sleeping sickness, is a vector-borne parasitic disease. The protozoa belong to the genus *Trypanosoma* and are transmitted to humans by the bite of the tsetse fly (*Glossina* genus). These flies can be found in sub-Saharan Africa, thus 36 countries and about 60 million people are affected by HAT; the number of current cases was estimated at 30,000 in 2010. *T. b. rhodesiense* and *T. b. gambiense*, two human pathogenic subspecies that cause either an acute or a chronic infection show similar clinical stages after transmission. First the parasites proliferate in the blood and the lymphatic system. In the second stage, the parasites cross the blood–brain barrier to infect the central nervous system (CNS). Without medical treatment of this severe stage, patients fall into a coma and finally die. Unfortunately, therapy is limited to the low number of currently available drugs and, due to spreading drug resistance, to their effectiveness. Based on a structure–activity relationship analysis, we recently found

the 7-morpholino-4-quinolone-3-carboxamide derivative **1** as a promising new lead compound with high in vitro activity against *T. b. brucei* (IC₅₀=47 nM) and *T. b. rhodesiense* (IC₅₀=9 nM) combined with low cytotoxicity against macrophages J774.1 (IC₅₀=57.0 μM). First biological experiments concerning the identification of the target and the mechanism of action indicated that **1** interferes with correct segregation of the kinetoplast resulting in a segregation defect. Furthermore, the mitochondrial topoisomerase II is not the only target for these new 4-quinolone-3-carboxamides in contrast to classic quinolone antibiotics like ciprofloxacin.^[1] Unfortunately, no in vivo data could be obtained so far because the 4-quinolone-3-carboxamides suffer from low water solubility derived from their layer lattice crystal structure. To solve this problem, we are pursuing two different approaches: 1) A preliminary formulation was developed to overcome the solubility problems by forming stable emulsions in aqueous solutions; 2) By means of structure modification, the future goal will be to increase the water solubility of these 4-quinolone-3-carboxamides without losing antitrypanosomal activity.



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P454

Fluorinated Phenylalkyl Ethers as Long-Acting Beta2 Adrenergic Agonists

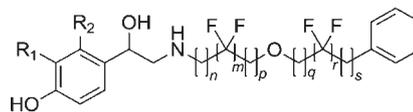
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The search for new ultra long acting β₂-adrenoreceptor agonists (LABA) has become a very active area in the last years, and some compounds are currently under registration or in advanced development stage for a once daily dosing.^[1–3]

The design, synthesis, and in vitro/in vivo SAR of a fluorinated phenylalkyl ether series of aryl ethanolamine β₂ adrenergic agents (see figure) will be presented. All compounds are highly β₂/β₁ selective (assessed as the ratio between IC₅₀ values for relaxation of guinea pig tracheal rings and rat left atria). Some compounds exhibit good potencies and duration of action in the reversion of acetylcholine-

induced bronchoconstriction model in guinea pig. The effect of the adrenergic head, length of the alkyl chains, and position of the fluorine atoms on activity/duration will be discussed.



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P455

Synthesis and Anticancer Screening Studies of Benzhydrylpiperazine Carboxamides

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Cancer is the disease resulting from abnormal cells with abilities of uncontrolled dividing and invasion to other tissues through blood and lymph systems.^[1] Anticancer activities of piperazine-bearing compounds are often reported.^[2,3] Benzhydrylpiperazines are popular for their antihistaminic activities,^[4,5] in addition, their cytotoxic activities have recently advanced according to literature.^[6,7]

In this study, 25 compounds with *N*-substituted-4-[[4-(4-substituted)diphenyl)methyl]piperazine-1-carboxamide structures were prepared. Benzhydrylpiperazine, 4-chlorobenzhydrylpiperazine and 4,4'-difluorobenzhydrylpiperazine were synthesized by reflux of piperazine and suitable benzhydryl chlorides in alkali medium. Compounds were synthesized with reactions of benzhydrylpiperazine derivatives with suitable isocyanates in room temperature with triethylamine. Structures of compounds were clarified with IR, ¹H NMR, ¹³C NMR, mass spectroscopies and elemental analyses; also, their physical characteristics and *R_f* values on thin layer chromatography were determined.

In vitro cytotoxic activities were screened in comparison with camptothecin (positive control) and 5-fluorouracil (reference) by a sulphorhodamine B assay against breast cancer (MCF-7), hepatocellular carcinoma (HUH-7) and colorectal carcinoma (HCT-116) cell lines. In general, 4-chlorobenzhydrylpiperazine derivatives were more potent than other derivatives against all cell lines. The most potent compound against the HUH-7 cell line was *N*-(4-cyanophenyl)-4-[[4-(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (**25**; IC₅₀=1.29 μM). Additionally, the most potent compound against the MCF-7 cell line was *N*-(2,6-dichlorophenyl)-4-[[4-(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (**21**; IC₅₀=6.14 μM). Moreover, the most potent compounds against the HCT-116 cell line were *N*-

tert-butyl-4-(diphenylmethyl)piperazine-1-carboxamide (**2**; IC₅₀=1.01 μM) and *N*-(4-cyanophenyl)-4-[(4-chlorophenyl)(phenyl)methyl]piperazine-1-carboxamide (**25**; IC₅₀=1.81 μM).

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P456

A New High-Throughput Screening Test Measuring Artificial Permeability Coupled with P-Glycoprotein Interaction

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Inappropriate pharmacokinetics (PK) have been recognized as being one of the major factors leading to the withdrawal of new chemical entities (NCEs) from the drug development process. Therefore a large number of compounds have to be screened before matching one drug candidate disclosing good ADMET (absorption, distribution, metabolism, elimination, toxicity) properties during the early stage of drug discovery. In this context, high-throughput methods thus become a real need for early assessment of compound PK properties and, in particular, their ability to penetrate biological membranes. It is well known that passive permeability of compounds through membranes is of prime importance in bioavailability, but it is also recognized that the efflux protein P-glycoprotein (Pgp, MDR1, ABCB1) affects the ADME of a large variety of compounds. This protein is well known to be responsible for some resistance in chemotherapy treatment, by overexpressing itself in cancer cells.^[1] The Pgp is also naturally involved in the human body for biliary excretion, blood-brain barrier, and gastrointestinal track interference.^[2]

The aim of this study was to develop a high-throughput assay able to simultaneously predict passive permeability through the intestinal track and affinity of compounds for Pgp. The assay is based on a PAMPA (parallel artificial membrane permeability assay) technique developed to predict passive permeability through biological membranes, where a donor and an acceptor compartment are separated by a liquid artificial membrane. Depending on the nature of the artificial membrane, different biological barriers can be targeted.^[3–6] In this study, hexadecane has been used as an artificial membrane to mimic the passive diffusion through the intestinal track.^[3] Most in vitro assays that are used to determine the interaction between NCEs and Pgp are performed with cells such as Caco-2 or MDCK117-

8. In this assay, purified membrane vesicles from Sf9 (*Spodoptera frugiperda*) expressing a high level of human recombinant Pgp were also added in the donor or in the acceptor compartment with the addition of compounds inhibiting all other ATPase pumps present in the vesicles such as calcium or sodium/phosphate pumps.^[9] Permeability obtained in the presence and absence of efflux protein was compared with each other. Compounds which interact with the Pgp have a potential equilibrium with this protein which should lower the rate of permeability through the artificial membrane.

This assay has to remain simple and easy to use; this is why the compound concentrations were determined with a UV/Vis spectrophotometer. The obtained values were compared at first with well-known compounds with in vivo permeability and Pgp substrate data available. This assay makes it possible to obtain two pieces of information which are nowadays essential in the conception of new drug entities.

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P457

Click-Xyloside Derivatives—Stimulators of Glycosaminoglycan Biosynthesis

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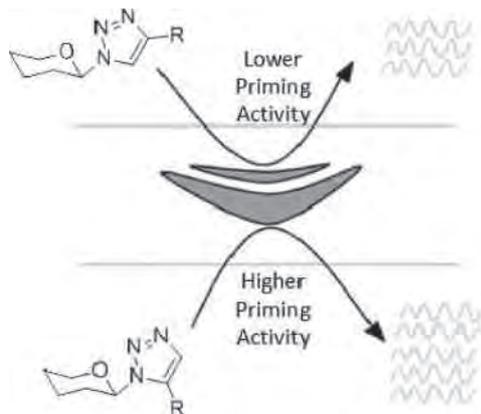
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Proteoglycans consist of a core protein and one or more glycosaminoglycan (GAG) chains. The biosynthesis of proteoglycans occurs in the golgi and follows a number of enzymatic modifications. Xylosylation is the first event in the assembly of GAG chains. Previous studies have found the addition of xylose residues attached to an aglycone, called xylosides, can induce the formation of GAG chains without the presence of a core protein. Alteration of the aglycone attached to the xylose can alter the priming activity of the molecule as well as the type, sulfation pattern, and molecular weight of the primed GAGs. The most commonly studied xylosides have been the *O*-xylosides. *O*-xylosides are often unstable, so alternative types of xylosides are currently being explored. A promising set of new xylosides, called click xylosides, are formed by copper(I)-catalyzed click reactions. These molecules are stable in culture and in vivo. Recent studies have examined how

these click xylosides induce GAG formation and how alteration of the aglycone can change the primed GAGs. However, copper-mediated click chemistry is only one form of click chemistry. This study serves to compare the priming capabilities of copper(I)-mediated click xylosides and ruthenium mediated click xylosides. The use of ruthenium in click chemistry results in a 1,5-xyloside orientation as compared to the 1,4-copper mediated click xylosides. This study shows that the ruthenium click xylosides tested have higher priming activity as compared to their copper click counterparts. However, while priming activity was increased, the type of GAG primed as well as the primed GAGs molecular weight, level of sulfation, and disaccharide profiles remained similar between the two groups.



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P458

Novel Approaches of Kinase Inhibition

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Most of the efforts in the development of kinase inhibitors concern compounds targeting the conserved ATP binding site, with notable successes reaching the market and helping patients. However, it has been recognized that allosteric inhibition of protein kinases is an attractive approach to address several issues that have arisen during the development of ATP-site-directed inhibitors, such as selectivity, resistance, and the identification of novel chemical matter. Examples will be presented using tailor-made screening approaches, structural biology and biophysical experiments to determine functional read-out for binding in order to identify novel allosteric kinase inhibitors. The particular focus of the presentation will be on insulin growth factor 1 receptor tyrosine kinase IGF1R, from the identification of ligand-pocket pair to relevance in an in vivo setting.

P459

Synthesis of Novel Pharmacological Inhibitors of HSP70 for the Treatment of Multiple Myeloma

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Since multiple myeloma (MM), a malignant disorder of plasma cells in the bone marrow, is still a remediless disease; the development of novel drugs is urgently needed.^[1] The approach chosen here makes use of the observations on the role of heat shock proteins (HSP) in MM.^[2] After HSP90 was addressed as a favorable anticancer drug target due to its role in oncogenic signaling and malignant growth, first clinical trials discovered an upregulation of HSP70 after treatment with HSP90 inhibitors. HSP70 takes over the function of the inhibited HSP90, which ultimately diminishes the cytostatic effect. Thus, dual depletion of HSP70 and HSP90 is expected to potentiate the apoptotic effect against cancer cells.^[3] Although many inhibitors of HSP90 have already been described, efficient and selective inhibitors of HSP70 are not yet available. Supported by molecular modeling, a novel potential binding site of the HSP70 protein was addressed with a newly designed compound series. In first biological studies, these compounds exhibited sub-micromolar EC₅₀ values against malignant myeloma cells, whereas no reduction in the cell viability could be observed with control cells. Western blot analyses strongly indicate an HSP70-selective mechanism for these compounds.

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P460

Critical Evaluation and Comparison of Capillary Electrophoresis, Liquid Chromatography and Surface Plasmon Resonance to Study Drug–Plasma Protein Interactions

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Drug–plasma protein interaction is a common process that every drug encounters once it is absorbed and reaches the blood compartment, which is composed of several proteins. Only the free drug

fraction is available to diffuse from the vascular system to organs or tissues where it interacts with therapeutic targets. Therefore, the extent of drug binding to plasma proteins has a significant impact on its pharmacokinetics and pharmacodynamics. The need for drug–plasma protein binding studies in discovery and preclinical development stages is thus essential for the prediction and understanding of pharmacokinetics as well for the design of optimal dose prescriptions. In this work, a systematic comparison of three analytical methods, namely capillary electrophoresis (CE), liquid chromatography (LC), and surface plasmon resonance (SPR), was performed for quantitative assessment of binding strengths of small molecules interacting with plasma proteins. In capillary electrophoresis, frontal analysis (CE/FA) was used to analyze drug–protein interactions and to obtain the affinity constant and stoichiometry of the studied interaction. High-performance affinity chromatography (HPAC) in zonal elution mode was then evaluated. This analytical tool allowed rapid binding percentage determinations and was particularly appropriate for screening experiments. Finally, an SPR-based biosensor was used as a suitable method for the quantitative assessment of strong noncovalent interactions. Affinity constants or binding percentages were obtained from each method and compared to elucidate the pros and cons of the selected methodologies in terms of performance, information obtained, time required to get a binding information, sample consumption, and cost.

P461

A Designed Dimeric Dipeptide Mimetic of Nerve Growth Factor Active on Animal Models of Parkinson's Disease and on Stroke in Rats

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Nerve growth factor (NGF) binds to TrkA receptor and initiates receptor dimerization, phosphorylation and a cascade of signaling events, including PI3K/Akt and MAPK/ERK pathways, which play critical roles in neuronal plasticity, survival and nerve outgrowth. Correlations between alterations in NGF expression and/or function and mechanisms occurring in Parkinson's disease, Alzheimer's disease, aging and stroke suggest that TrkA agonists might have therapeutic potential.

To mimic NGF functions pharmacologically, we designed dimeric *N*-acyldipeptides mimicking loops 1 and 4. The mimetic bis(*N*-succinyl-L-glytamul-L-lysine) hexamethylenediamide, named GK-2, was designed on the basis of beta-turn sequence Asp93–Glu94–Lys95–Gln96 of loop 4, and the mimetic bis(aminohexanoyl-glycyl-L-lysine) hexamethylenediamide, named GK-6, was designed on the basis of sequence Lys32–Gly33–Lys34–Glu35 of loop 1. These sequences were chosen as the basis for modeling because they contain dipeptide fragments most exposed to solvent and so occupy the position geometrically most advantageous for interaction with the receptor. The preceding dipeptide fragments amino acid residues (Asp93 and

Lys32) were substituted by bioisosteres: the aspartic acid residue Asp93 was replaced by a succinic acid residue and the lysine residue was replaced by a aminohexanic acid residue. This substitution might increase resistance of molecules to proteolysis. The residue following the dipeptide fragment was represented by the alkylamide group. Because NGF interacts with the TrkA receptor in the dimeric form, agonistic activity was achieved by dimerizing the *N*-acyldipeptides by hexamethylenediamine. This spacer was optimal since the reduction in the length to pentamethylenediamine led to a decrease and then the reverse of activity, and the elongation did not enhance the biological activity. The mimetics designed were synthesized by methods of classical peptide synthesis in solution including the methods of activated esters and azide method with the use of the strategy of Z/Boc protecting groups.

The compounds GK-2 and GK-6 stimulated tyrosine phosphorylation of TrkA, but not TrkB, receptor. The dipeptide GK-6 demonstrated slight neuroprotective activity *in vitro* at concentrations of 10^{-5} – 10^{-8} M and was able to induce differentiation of PC-12 cells. The dipeptide GK-2 prevents glutamate- and H₂O₂-induced neuronal cell death at concentrations of 10^{-5} – 10^{-9} M, but does not provoke neurite outgrowth in PC-12 cells. It was shown that PI3 kinase inhibitor LY294002, but not MAP kinase inhibitor PD9859, abolishes neuroprotective effects of GK-2 *in vitro*. Administration of GK-2 in rats (0.1–1 mg/kg ip) decreases infarct volume in the transient middle cerebral artery occlusion model of stroke and modulates symptoms in rodent models of Parkinson disease. Thus, the designed dipeptide mimetics not only establish a powerful platform for dissection of the physiological roles of NGF and TrkA receptor but also provide effective treatments for neurodegenerative diseases and stroke.

P462

Structure-Based Discovery of Novel DNA Gyrase B Inhibitors

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The emergence of bacterial resistance to most of the clinically used antibiotics and the urgent need for the discovery of potent antibacterials with broad spectrum of efficacy and improved safety profile has revived the research in this field.^[1,2] A challenge for the development of novel and effective antibacterial agents are suitable and validated targets.^[3] One of the well-established targets is DNA gyrase B, a topoisomerase with ATP-ase activity. In the absence of ATP, DNA gyrase catalyzes only the relaxation of supercoiled DNA but not the introduction of negative supercoils.

In our ongoing effort to identify low-molecular-weight ligands with inhibitory activity towards DNA gyrase B, we used a structure-based fragment design approach. Starting from the available structural information about the binding mode of the natural product inhibitors, cyclothialidine^[4] and clorobiocin,^[5] we identified a novel series of indolinone-2-ones,^[6] 2-2-amino-4-(2,4-dihydroxyphenyl)

thiazoles,^[7] rhodanines,^[8] and more recently, 4'-methyl-N2-phenyl-[4,5'-bithiazole]-2,2'-diamine^[9] inhibitors of gyrase B with low micromolar inhibitory activity. These inhibitors were subsequently extensively characterized by various biophysical techniques (differential scanning fluorimetry, surface plasmon resonance and microscale thermophoresis). The binding mode of the most potent inhibitor **18** was revealed by high-resolution X-ray crystallography, confirming our initial in silico binding model. The crystal structure of the complex protein G24 and inhibitor **18** provides valuable information for further optimization of this novel class of DNA gyrase B inhibitors. Furthermore, the high resolution of the complex structure allowed for the placement of the Gly97–Ser108 flexible loop and its role in binding of this class of compounds. This result nicely corroborates with the highest increase in thermal stability upon the formation of the complex with compound **18** as determined by differential scanning fluorimetry.

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P463

Synthesis of New Steroid– β -Lactam Hybrids via Palladium-Catalyzed Aminocarbonylation

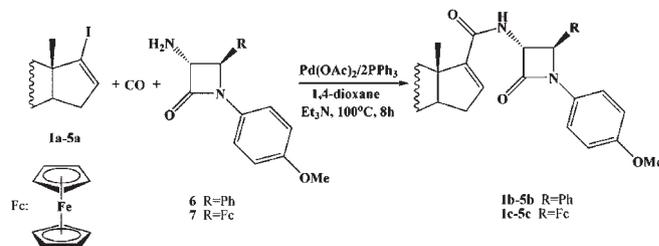
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The synthesis of a great number of molecular hybrids or ‘chimeras’ derived from steroids and other biomolecules or drugs have been reported in the literature. Steroidal β -lactams were isolated from the plants *Pachysandra terminalis* and *Pachysandra procumbens*. The latter group of compounds showed antiestrogen-binding site inhibitory activity. There are also some reports on some steroid– β -lactam hybrids prepared synthetically, including cholestane, androstane, estrane and cholane conjugates. Although the biological activity of most of the compounds has not been revealed, a number of 1,2,3-triazol-linked β -lactam–bile acid conjugates exhibited significant antifungal and moderate antibacterial effect. In the present work, the enantiomerically pure *trans*-3-amino- β -lactams **6** and **7** were coupled to the steroidal core **1a–5a** via an amide bond constructed by palladium-catalyzed carbonylation reaction in order

to obtain steroid– β -lactam hybrids **1b–6b** (see scheme). Amino- β -lactam derivatives have been used for the first time as nucleophiles in the aminocarbonylation reactions.



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P464

Synthesis of 5-Bromomethyl-4-nitroimidazoles and Their Use during Lead Optimisation of SN29966: A Hypoxia-Activated Prodrug of an Irreversible Pan-HER Inhibitor

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Hypoxia occurs in most solid tumours and is associated with disease progression, resistance to conventional therapies and poor patient outcome. We have developed SN29966, a nitro methylaryl quaternary (NMQ) ammonium salt, as a prototype hypoxia-activated prodrug that fragments to release the irreversible pan-HER inhibitor, SN29926 upon one-electron (1e) reduction selectively under hypoxia.^[1]

Herein, we describe lead optimisation studies around SN29966 including synthesis of analogues with variations of the pan-HER “effector” and the 4-nitroimidazole “trigger”, particularly seeking to influence the 1e reduction potential of the trigger nitro group and therefore the fragmentation rate and hypoxia-selectivity of the prodrugs. A series of 5-bromomethyl-4-nitroimidazole trigger precursors bearing electron-donating and electron-withdrawing substituents at the N-1 and C-2 positions were synthesised. New methodologies were developed including the efficient radical bromination of 5-methylimidazoles using NBS under light initiation and a versatile Pd-catalysed cyanation of 2-bromoimidazole derivatives.

Solubility, stability, 1e reduction potential, fragmentation rate and hypoxic cytotoxicity ratios of the resultant prodrugs in representative cancer cell lines will be discussed. Pharmacokinetic screening and anti-tumour activity in NIH-III mice bearing erlotinib-resistant NSCLC (H1975) xenografts recommended SN32807 as a lead candidate for clinical evaluation.



Figure 1. SN29966 bearing a “trigger” (blue) and trigger precursors, 5-bromo-methyl-4-nitroimidazoles.

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P465

One-Pot Synthesis and Anticancer Effect of Novel 9-Anilinoacridine Derivatives

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A series of novel 9-anilinoacridine derivatives were synthesized for anticancer and structure–activity relationship studies using a new, highly efficient one-pot derivatization of 9-aminoacridine (9-AA) at the 9-amine position by simple reductive amination or S_NAr reactions, yielding libraries of novel substituted *N*(9)-benzylaminoacridines and *N*(9)-anilinoacridines correspondingly. Such a unique method allows fast formation of aniline and benzyl tethers with electron-withdrawing (EW) groups in 9-AAs. Several of these new 9-anilinoacridine derivatives exhibited significant cytotoxicity in inhibiting growth of the following cancer cell lines: NSC-34 (mice spinal cord neuroblastoma), LNCaP (human prostate adenocarcinoma), PC-12 (rat pheochromocytoma), PC-3 (human prostatic carcinoma), HepG2 (human liver carcinoma) and D122 (mouse Lewis lung carcinoma). The compound GG-59 was most effective (86% inhibition of the cell growth) and most potent (5 μ M) against all tested cell lines. Additionally, GG-59 was approximately threefold more effective than its two parent analogues 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA) and amsacrine in inhibiting growth of the cells. The possible toxic effect on viability of normal, high differentiated cells (rat L6 myotubes) was also tested. GG-59 did not show any toxic effects on the L6 myotubes, compared to significant toxicity of both parent analogues. The inhibitory effect of GG-59 on HepG2 cells was most sufficient in comparison to other cells. In vitro investigations of the mechanism of action of GG-59 and other 9-anilinoacridine derivatives are currently

underway in our laboratories. These findings indicate that GG-59 can be used as a potential prototype molecule for the development of novel anticancer drugs, especially against liver cancer.

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A Novel Series of Orexin Receptor Antagonists with a Distinct Effect on Sleep Architecture

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Orexin A and B (hypocretin 1 and 2) are two neuropeptides produced in discretely localized neurons in the hypothalamus, with widespread projections to various brain regions. These peptides activate two G-protein coupled receptors Ox1 and Ox2, generally leading to excitatory postsynaptic effects. The orexin system plays a role in the regulation of the sleep–wake cycle, feeding, and reward seeking. It therefore was suggested that orexin receptor antagonists could be useful for the treatment of related disorders, in particular insomnia. Positive proof of concept clinical studies in primary insomnia were reported with four structurally diverse dual orexin receptor antagonists (Almorexant, Suvorexant, SB-649868 and MK-6096).

Here, we present the discovery, optimization, and preclinical characterization of a novel class of orexin receptor antagonists that induce sleep in rodents. Interestingly, they exhibit a remarkably distinct effect on the sleep architecture determined by EEG when compared to previously reported clinical candidates.

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Carbaborane-Containing Building Blocks for Selective Antitumour Activity in Functionalised Neuropeptide Y Derivatives

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Boron neutron capture therapy (BNCT) has the potential to overcome problems of current standard cancer therapies such as a variety of undesired side effects and a high mortality rate. However, for successful BNCT treatment, it is required to selectively accumulate a high number of ^{10}B nuclei in cancer cells (10–30 μg per gram tumour tissue). After thermal neutron irradiation the ^{10}B isotope decays

into a ^4He and ^7Li atom with high kinetic energy (Figure 1A). This cell-destroying particle radiation is limited to the cell (tumour cell) where it is formed.^[1]

Therefore, we focused on the conjugation of the metabolically stable carborane clusters containing ten boron atoms per molecule with a tumour-selective peptide ([Phe7,Pro34]-NPY, Figure 1B). The [Phe7, Pro34]-modified NPY displays a high selectivity towards the human Y_1 receptor subtype, which is overexpressed in 90% of breast tumours and 100% of metastases, whereas normal breast tissue mainly expresses the Y_2 receptor. We synthesised different carborane-containing building blocks that could be coupled to the [Phe7,Pro34]-NPY by solid-phase peptide synthesis. Despite introduction of the cluster, nanomolar binding affinities and activation of the receptor were observed. Receptor subtype-specific uptake of the boron-containing ligand/receptor complex could be demonstrated by internalisation studies.^[2]

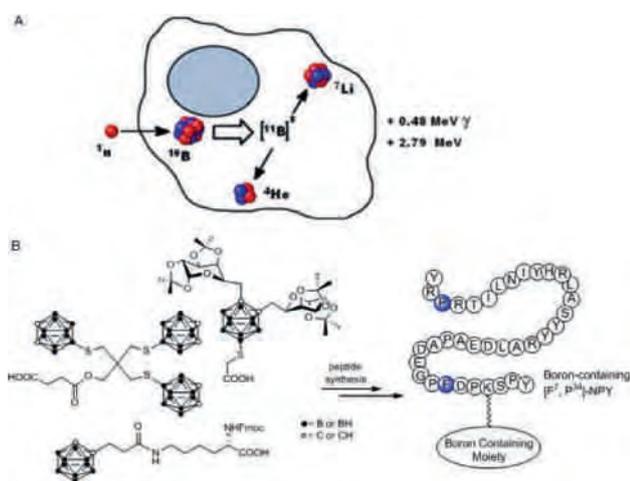


Figure 1. A) Neutron capture of a ^{10}B core and subsequent decay. B) The carborane derivatives (left) are incorporated into [Phe7, Pro34]-NPY (right). L-Lysine (K) is a promising amino acid to be modified in this peptide.

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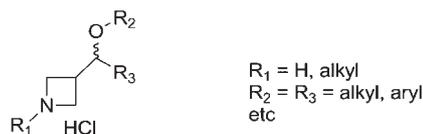
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Exploration of Azetidine Derivatives as Triple Re-uptake Inhibitors

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Major depressive disorder (MDD) is a common and serious illness with the potential of becoming the leading cause of disability worldwide. Pathophysiologically, the cause of depression is commonly associated with monoamine neurotransmitter (serotonin (5-HT), norepinephrine (NE) and dopamine (DA)) deficiency in the brain, and a number of antidepressants aim to increase the levels of these neurotransmitters in the synapse. Among various monoaminergic strategies for maintaining the concentration of neurotransmitters, blocking pre-synaptic 5-HT, NE and DA transporters (SERT, NET and DAT) to re-uptake inhibition of neurotransmitters into nerve terminals has been an important strategy in modern antidepressant therapy.^[1] Although many kinds of re-uptake inhibitors such as selective serotonin re-uptake inhibitors (SSRIs) and serotonin norepinephrine re-uptake inhibitors (SNRIs) are commercially available for the treatment of major depression, they may take several weeks of treatment before symptoms improve and some inhibitors reveal a few side effects such as insomnia and sexual dysfunction.^[2] One strategy to improve efficacy and/or reduce delay to onset of action of them is the addition of a dopamine component to SSRIs or SNRIs. This is the concept of triple re-uptake inhibition which blocks synaptic re-uptake to all of 5-HT, NE, and DA. A few classes of triple re-uptake inhibitors (TRIs) including DOV 216 303^[3] and GSK 372 475^[4] showed positive phase II clinical effect, but there are no triple re-uptake inhibitors available yet in the market, and combination and multiple drugs therapy to inhibit the re-uptake of 5-HT, NA and DA may raise some problems of pharmacokinetics. Therefore, triple re-uptake inhibitors as a single molecule are expected to be the next generation of antidepressant and are still desirable. We will present an exploration of novel azetidine derivatives that show inhibition of 5-HT, NE as well as DA transporters, including molecular design through mapping of the pharmacophore by a computer-aided technique, syntheses, and screening results by a fluorescence-based HTS uptake assay.



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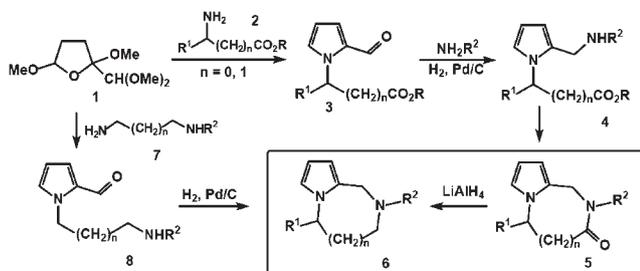
Synthesis of Novel Pyrrolo[1,2-*a*]pyrazines and Pyrrolo[1,2-*a*][1,4]diazepines Derivatives and Their Anxiolytic and Antidepressant Activity

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The number of depressions, pathologic anxieties and cognitive function disorders is constantly increasing due to lifetime and life rate increase. Therefore, the search for new drugs for these diseases is an important task. The various 1,2-dihydro- and 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazines were shown to have pronounced neuropsychotropic activity.^[1] The purpose of this study was the synthesis and study of antidepressant and anxiolytic activity of novel pyrrolo[1,2-*a*]pyrazine and pyrrolo[1,2-*a*][1,4]diazepine derivatives, which have structural similarity with the previously synthesized active compounds and known drugs of this type.

1,2-Dihydropyrrolo[1,2-*a*]pyrazin-3(4*H*)-ones and 1,2,4,5-tetrahydro-3*H*-pyrrolo[1,2-*a*][1,4]diazepin-3-ones (**5**) were formed on the basis of 2,5-dimethoxy-2-dimethoxymethyltetrahydrofuran (**1**). The reactions of this compound with amino acids or their esters (**2**) gave (2-formyl-1*H*-pyrrol-1-yl)carboxylic acids and their esters (**3**) which then reacted with amines under reductive conditions to give the amino acids or amino esters **4**. In case of synthesis using amino acids, the corresponding compounds **4** (R=H) were transformed into esters **4** (R=Me). The latter were successfully cyclized into target lactams **5**. Lithium aluminum hydride reduction of bicycles **5** led to 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazines and 2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*a*][1,4]diazepines (**6**). These compounds were also obtained by a two-stage scheme from cyclic acetal **1**. Reaction with *N*-substituted diamines **7** led to aminoaldehydes **8**, which were transformed into target bicyclic amines **6** by catalytic hydrogenation.



Neuropsychotropic activity of the synthesized compounds (**5** and **6**) in vivo has been studied in outbred white male rats weighing 200–250 g with intraperitoneal injection using standard validated methods in doses of 7 mmol/kg (1–2.5 mg/kg). The Vogel conflict situation model was used for anxiolytic activity evaluation, which is based on the conflict of drink motivation and electropainful irritation. The daytime tranquilizer medazepam was used as a reference preparation in dose of 10 mg/kg. Porsolt and Nomura forced swimming tests were employed for antidepressant activity evaluation. The famous tricyclic antidepressant amitriptyline was used as a reference preparation in dose of 10 mg/kg. A study of pyrrolo[1,2-*a*]pyrazine and pyrrolo[1,2-*a*][1,4]diazepine derivative (**5** and **6**) activity showed that some of these compounds possess high anxiolytic and antidepressant activity that is comparable to or greater than the activity of reference preparations. The structure–activity relationship as well as target compound synthesis will be discussed in detail.

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Design, Synthesis and Pharmacological Characterization of the First Selective Inhibitors of Neurotrypsin

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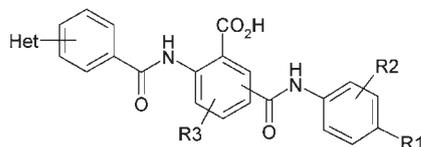
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Neurotrypsin is a neural serine protease, identified and characterized in the nervous tissue,^[1] whose protease domain exhibits ca. 40% amino acid identity to plasmin, trypsin, neuropsin and tPA. Structure predictions characterize the 875 aa human neurotrypsin as a secreted serine protease with a proline-rich basic segment, one Kringle domain followed by four scavenger receptor cysteine-rich repeats and the serine protease domain. The sole neurotrypsin target identified thus far is agrin, a heparan sulfate proteoglycan widely expressed in the nervous system and in many extraneural organs,^[2] which plays an essential role in the development and maintenance of neuromuscular junctions (NMJ).^[3] Agrin-deficient mice die at birth because of nonfunctional NMJs and consequent respiratory failure. Also, recent studies suggest a role for agrin, and its 22 kDa C-terminal fragment (CAF) produced by neurotrypsin cleavage, in the formation and/or maintenance of central excitatory synapses.^[4]

Transgenic mice overexpressing neurotrypsin (Sarco mice^[5]) show excessive agrin cleavage, as assessed by the increase of the biomarker CAF in plasma, resulting in fragmentation and reduction of nerve terminals and NMJs, degenerative loss of skeletal muscle mass and strength, and histopathological alterations closely

mimicking those observed in sarcopenia. It is readily apparent that specific inhibitors of neurotrypsin would constitute a therapeutically useful tool for all malfunctions of cholinergic synapses due to alterations of the delicate equilibrium between agrin and neurotrypsin, including sarcopenia, motor neuron diseases and neurodegenerative disorders where destruction of cholinergic synapses is involved.

As none of the known serine protease inhibitors has any significant activity against neurotrypsin, novel potential inhibitor molecules were designed by rational modification of known protease inhibitors and virtual screening of commercial libraries using putative pharmacophore models. Also, a neurotrypsin homology model was built from the X-ray structures of similar serine proteases to help investigate the binding mode of inhibitors. Identification of a few initial hits with potency in the low micromolar range allowed the building of a predictive QSAR model which accelerated the lead discovery phase and provided full lead compounds that inhibited neurotrypsin activity, with IC_{50} values in the high nanomolar range and showing more than 100-fold selectivity against a panel of seven different serine proteases. Preliminary *in vivo* evaluation of two lead compounds confirmed that intraperitoneal administration of these inhibitors to C57/BL6 mice was able to reduce the plasma concentration of CAF, without causing any major toxic effects.



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Stereoselective Divergent Synthesis of Four Diastereomers of Pachastrissamine (Jaspine B) and Its Sphingosine Kinase Inhibitory Activities

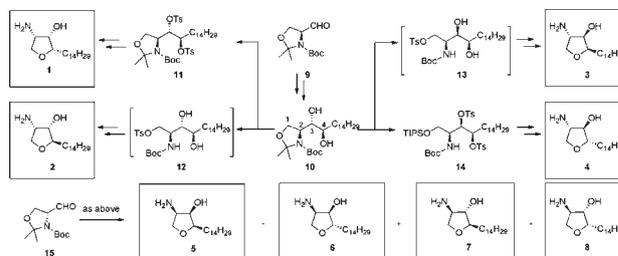
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Pachastrissamine (**1**), a naturally occurring anhydrophytosphingosine derivative, was isolated from the Okinawan marine sponge, *Pachastrissa* sp. (Scheme 1). Pachastrissamine exhibits marked sub-micromolar cytotoxicity against several cancer cell lines, and the cytotoxic potency is dependent on the stereochemistry of the tetrahydrofuran moiety. In view of the biological relevance of the pachastrissamine stereochemistry, a facile synthetic route to a series of pachastris-

samine stereoisomers has been required. In this presentation, we will report the stereoselective synthesis and biological activities of eight pachastrissamine stereoisomers **1–8**.

Divergent synthesis of four pachastrissamine diastereomers **1–4** began with a common diol substrate **10**, which was easily prepared from *S*-Garner's aldehyde **9** in two steps. The other enantiomers **5–8** were prepared from *R*-Garner's aldehyde **15** using this successful approach (Scheme 1).



Scheme 1. Stereoselective synthesis of pachastrissamine stereoisomers **1–8**.

Overexpression of sphingosine kinases (SphKs) in various human tumors has been reported, which catalyze sphingosine phosphorylation to form sphingosine-1-phosphate, thereby impairing the efficacy of chemotherapy. We evaluated naturally occurring pachastrissamine and all its stereoisomers for SphK inhibitory effects (Table 1). Among eight pachastrissamine stereoisomers **1–8** and a reference *N,N*-dimethylsphingosine **16**, (*2R,3S,4R*)-isomer **7** and (*2S,3S,4R*)-isomer **8** exhibited the most potent inhibitory activity against SphK1 and SphK2, respectively.

Table 1. IC_{50} values (μM) of sphingosine kinase inhibition.

Compd	SphK1	SphK2	Compd	SphK1	SphK2
1	4.6	6.6	6	2.1	6.2
2	3.9	15.8	7	0.59	1.8
3	2.1	6.1	8	0.94	0.48
4	3.0	2.2	16	2.8	13.7
5	2.7	10.5			

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Discovery of a New Heterocyclic Scaffold Present in Potent In Vitro and Ex Vivo Apoptosis Inhibitors

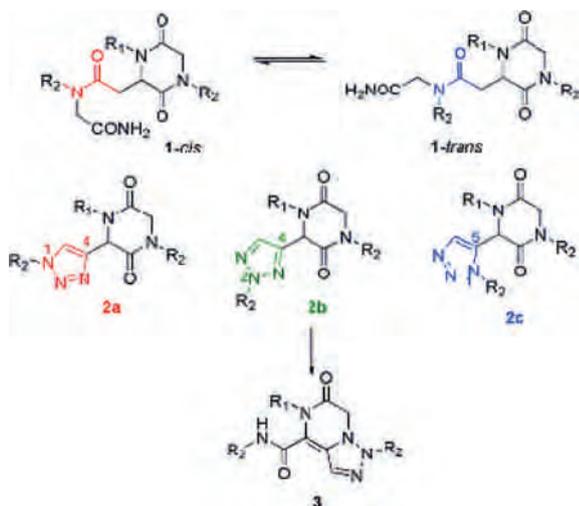
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Apoptosis is a biological process relevant to human disease stated that is regulated through protein-protein complex formation.^[1] In this context, the apoptosome is a multiprotein complex that is of interest for the development of apoptotic modulators.^[1] We have previously reported a peptidomimetic compound bearing a 3-substituted-piperazine-2,5-dione moiety as a potent apoptotic modulator.^[2] Structural studies of this compound showed the presence of *cis/trans* isomers of the exocyclic tertiary amide bond in slow exchange, which should be of high relevance for off-target interaction in front of the biological target.^[3]

This information encouraged us to perform an isosteric replacement of the amide bond by a 1,2,3-triazole moiety, where different substitution patterns would mimic different amide rotamers. The syntheses of these restricted analogues have been carried out using the Ugi multicomponent reaction followed by an intramolecular cyclization. Unexpectedly, for one of the proposed structures, a previously non-reported bicyclic compound was formed.^[4]

All of the compounds were shown to efficiently inhibit apoptosis, in vitro and in cellular extracts, with slight differences for the corresponding regioisomers. Noticeably, compound bearing the new scaffold showed the highest inhibitory activity. Moreover, computational studies also support the hypothesis that the inhibition mechanism is due to the binding to Apaf-1.



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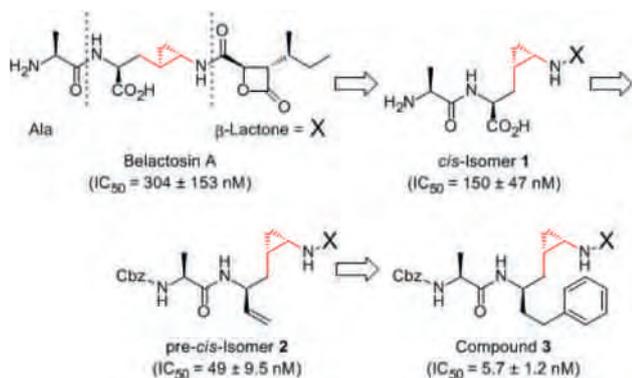
Structure–Activity Relationship Study of an Unnatural *cis* Isomer of Belactosin A, a Potent Proteasome Inhibitor

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Proteasome is a multicatalytic proteinase complex which works on the turnover of a large portion of intracellular proteins. In 2003, for the first time as a proteasome inhibitor, bortezomib was approved by FDA for the treatment of patients with relapsed or refractory multiple myeloma, and many proteasome inhibitors have been studied as potent anti-cancer drug candidates so far.

Belactosin A is a natural product isolated from *Streptomyces* sp.^[1] that has anti-proliferative activity in human cancer cell lines due to the proteasome inhibition.^[2] Previously, we synthesized stereo- and regioisomers of Belactosin A and found that *cis* isomer 1 of belactosin A has a somewhat greater proteasome inhibitory activity than belactosin A itself,^[3] and their synthetic precursors 2, having hydrophobic substituents, are much more potent (see scheme).^[4]



We have newly designed and synthesized a series of *cis*-belactosin A derivatives and found a highly potent proteasome inhibitor **3**, which inhibits the chymotrypsin-like activity of proteasome as strongly as bortezomib (IC_{50} =4.5 nM). Interestingly, the antiproliferative activity of these belactosin A derivatives are less than those expected from their proteasome inhibitory activity. We have investigated the stability and the reversibility of the proteasome inhibition of these compounds. These experiments suggested that these compounds are unstable in cells and inhibit proteasome activity in a reversible manner, while they covalently bonded to the proteasome. We will present the details of these results.

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Semi-synthetic Maslinic Acid Derivates with Promising Antineoplastic Activity

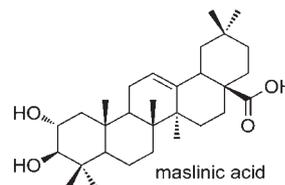
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The triterpene maslinic acid occurring in olive-pomace oil is a well-known natural product. Several groups established significant biological and medicinal effects for this compound, among them anti-oxidant,^[1] anti-inflammatory,^[2] anti-microbial, and anti-viral activities.^[3] During the last decade a pronounced interest arose in the antitumor effects^[4] of maslinic acid and derivatives; only a few studies^[5] on their mode of action can be found in literature.

Inspired by these interesting observations, we started modifying maslinic acid to obtain information about the structure-dependent mechanism of cell death induction. In addition, we were interested in accessing derivatives more active than parent maslinic acid and finding compounds showing a high tumor control/selectivity.

For the antitumor studies, nine different tumor cell lines and several non-malignant fibroblasts cells have been used, applying the well-established SRB-test. Additional AO/PI, Annexin V and cell cycle investigations were conducted for mechanistically studies. The synthesis of the new compounds as well as the results of the biological evaluation will be presented.



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Discovery of Imidazolidine-2,4-diones as Selective NPY Y2 Receptor Antagonists

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Neuropeptide Y (NPY) is a highly conserved 36-amino-acid peptide that belongs to the pancreatic polypeptide (PP) family and was first isolated from mammalian brain in 1982 (Tatemoto, **1982**). NPY is one of the most abundant neuropeptides in the mammalian central and peripheral nervous systems, and controls a wide spectrum of basic physiological functions. NPY strongly stimulates food intake, affects blood pressure and cardiovascular function, induces anxiety, affects circadian rhythms, and controls certain aspects of endocrine hypothalamic and pituitary functions (Heilig and Widlerlöw, **1995**; Thorsell and Heilig, **2002**). At the cellular level, NPY exerts its biological effects through an interaction with a portfolio of G-protein-coupled receptors. Presently, eight receptors for NPY have been described—Y1, Y2, Y3, Y4, Y5, Y6, Y7, and Y8. The Y3, 6, 7, and 8 receptors are either not functional or widely spread in non-human species. A wide range of human genetic, clinical, and preclinical evidence has accumulated supporting the relevance of NPY and Y2 receptors to psychiatry. We have demonstrated that intra-amygdala-injected NPY and the Johnson & Johnson Y2 antagonist BSD437 block the expression of cued conditioned freezing in mice. Also, intra-amygdala NPY blocked fear-potentiated startle in mice (Fendt, **2009**). NPY Y2 receptors are presynaptic autoreceptors, and

their activation results in suppression of extracellular NPY release (Naveilhan et al., 1998). Consequently, blockade of Y2 receptors is expected to produce elevated synaptic NPY levels which, in turn, should activate postsynaptic Y1 and Y5 receptors. Thus, augmentation of postsynaptic NPY-ergic transmission in the fear/anxiety/affect neurocircuitry may produce beneficial therapeutic effects in anxiety and mood disorders.

A GTPγS antagonist mode HTS was performed with the compounds of the Novartis compound collection using cells expressing recombinant human NPY Y2 receptors. This assay gave rise to hits based on imidazolidine-2,4-diones as interesting starting points towards the discovery of novel NPY Y2 receptor antagonist-based therapeutics. The subsequent hit-to-lead chemistry yielded the potent and selective NPY Y2 receptor antagonist NVP-833. This compound has an IC₅₀ value of 15 nM (*h*NPY-Y2 GTPγS) and a *K_i* value of 14 nM (*h*-rec binding assay). The preclinical profiles and SAR of NVP-833 and derivatives in the imidazolidine-2,4-dione series will be presented.

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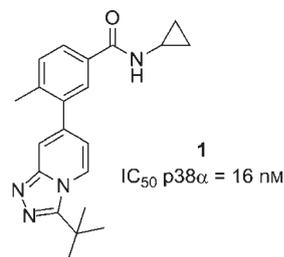
Novel Triazolopyridylbenzamides as Potent and Selective p38α Inhibitors: Design, Synthesis, SAR and In Vivo Anti-inflammatory Activity

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The pathophysiological consequence of excessive production of TNFα and IL-1β is thought to be responsible for the progression of many inflammatory diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease.^[1-4] The proven ability of p38α MAP kinase to efficiently regulate both the release and the activity of these pro-inflammatory cytokines has attracted numerous pharmaceutical companies into pursuing p38α inhibitors, primarily as novel anti-inflammatory drugs.^[5] However, although initial preclinical results seemed to be very promising,^[6,7] resulting in the advancement of several candidates into the clinic,^[8] recent reports on clinical trials performed with the most advanced compounds in rheumatoid arthritis have revealed limited efficacy.^[9] Currently, the potential use of such molecules in other indications is under study.^[10]

In this poster, a new class of p38α inhibitors based on a biaryl-triazolopyridine scaffold was investigated. X-ray crystallographic data of the initial lead compound **1** cocrystallised with p38α was crucial in order to uncover a unique binding mode of the inhibitor to the hinge region via a pair of water molecules. Synthesis and SAR was directed towards the improvement of binding affinity, as well as ADME properties for this new class of p38α inhibitors and ultimately afforded compounds showing good in vivo efficacy.



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New Advances in Neurodegenerative Diseases Structure-Based Drug Discovery

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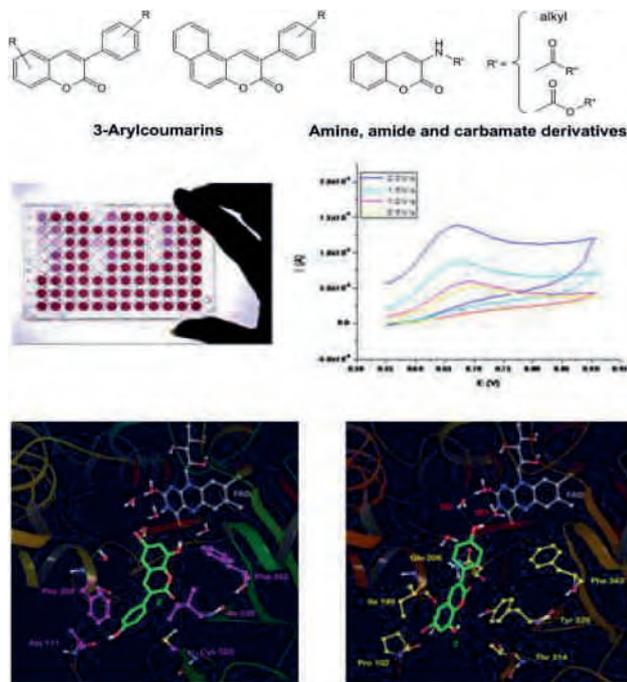
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With the significant increase of life expectancy of populations in societies today, the importance of the discovery of drugs associated with neurodegenerative diseases (ND) has emerged. Therefore, ND are an important topic in medicinal chemistry. The development of effective neuroprotective therapies that slow down or stop disease progression in the earliest stages is one of the main goals of the researchers in this area. The present communication provides an overview about the potential of different substituted coumarins as inhibitors of enzymatic systems (AChE, MAO-A, and MAO-B) and as protective agents from oxidative processes, both

involved in ND. As pathologies with multiple pathogenic factors, in the last years, the therapeutic sources have focused on the multitargeting strategies.



Coumarins are an important family of natural and/or synthetic compounds that occupy an important place in the area of natural products and organic chemistry.^[1-3] Some synthetic 3-arylcoumarins already proved to be very potent and selective MAO-B inhibitors. Based on these results, and with the aim of finding multitarget inhibitors, we designed, synthesized and evaluated new series of differently substituted coumarins as potential AChE, MAO-A, and MAO-B inhibitors, and as antioxidative agents. Different activities can be modeled according to the substituents presented on the coumarin scaffold. The synthetic routes and experimental results will be reported in the communication.

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P478

Discovery of Novel Natural Compound-Based Scaffolds as New Potent and Selective Adenosine Receptors Ligands

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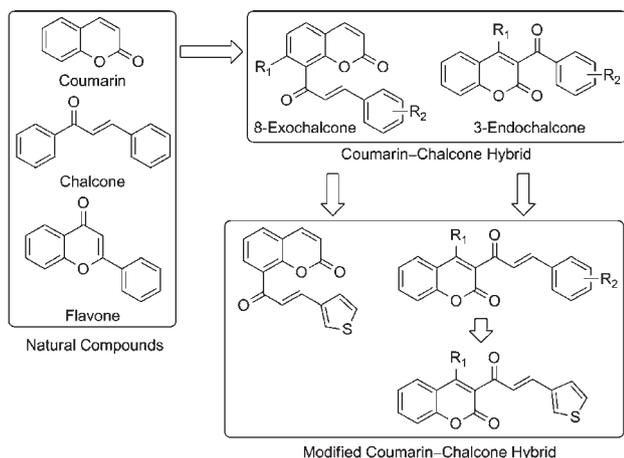
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The adenosine receptors (AR) are a class of G-protein-coupled receptors (GPCRs) with adenosine as endogenous modulator. Therefore, many physiological functions are controlled through the binding of adenosine to any of the four different types of AR (A₁, A_{2A}, A_{2B}, A₃), changing the levels of the second messengers such as diacylglycerol (DAG) or the cyclic 3',5'-monophosphate (cAMP) for signal transduction.^[1] For this reason, AR have been regarded as potential targets for the treatment of diseases related to these signal transductions pathways.^[2] While most of the ligands of adenosine have a purine-type structure, there are also some non-purine ligands that bind to these receptors, among which we can find flavonoid-type derivatives.^[3] The affinity of flavonoids and other phytochemicals to adenosine receptors suggests that a wide range of natural substances in the diet may potentially block the effects of endogenous adenosine. On the other hand, coumarin (flavone isosteres) and chalcones (open flavones) are natural and synthetic origin compounds that show a wide range of pharmacological activities including anticancer, anti-inflammatory, immunomodulatory, and immunosuppressive, among others.^[4,5]

With the aim of finding new drug candidates targeting the AR, and taking into account the structural similarities between flavonoids and the coumarin and chalcone scaffolds, coumarin–chalcone hybrid molecules have been synthesized, as well as compounds with key and bioisosteric modifications over this hybrid moiety. Binding affinity assays with the four different types of AR have been carried out as an initial screening, and promising results have been found. The synthesized compounds resulted in selectivity and potency either against the A₃ or the A₁ receptors. Synthetic procedures and biological results, as well as structure–activity relationships, will be presented.



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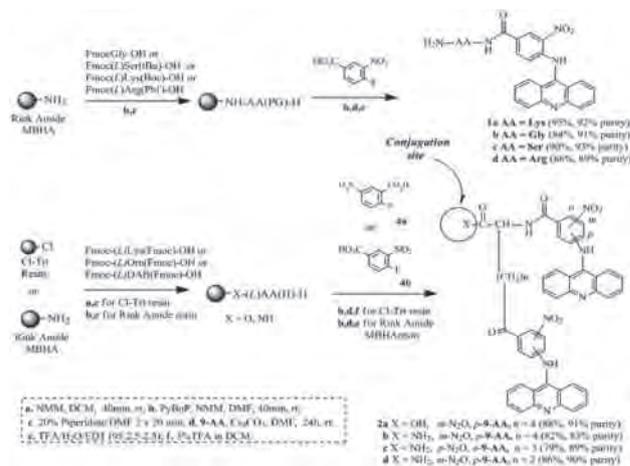
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SPOS Route to Novel Anticancer 9-Anilinoacridine Peptidyls

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A highly efficient derivatization of medicinally important 9-aminoacridine (9-AA) at the amine position using an S_NAr reaction on solid phase is described. The resulted 9-anilinoacridines (9-AnA), bis-9-anilinoacridines, and their peptidyl derivatives are easily obtained in good yields from accessible starting materials, rapidly generating novel potential DNA intercalators with variable spacer lengths and charged, polar, or hydrophobic residues at desired positions, which can increase binding affinity, conformation stability, intracellular transport and/or biological activity. The synthetic routes reported in this work are general and applicable, significantly expanding the scope of potential 9-AA anticancer hits. In vitro anticancer activity of representative compounds has been evaluated.



Scheme 1. SPOS of mono and bis peptidyl-9-anilinoacridine derivatives **1** and **2** by S_NAr .

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P480

Synthesis and Anticancer Activity of Novel Chromeno-imidazo[1,2-*a*]pyridines

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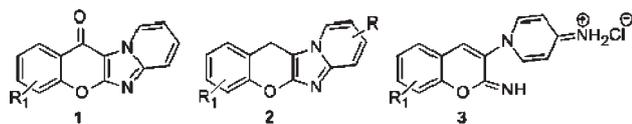
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Cancer is a devastating disease, and despite the huge efforts of the scientific community to come up with a cure, no real improvements were attained with the newer drugs marketed during the last decade. Limited effectiveness, toxicity, and eventual resistance remain a serious problem. The biological complexity of the disease usually requires the use of a combination of drugs acting on different receptors.^[1] Another plausible strategy involves the use of a single molecule capable of interfering with multiple altered pathways.^[2]

As part of a drug discovery collaboration program, a qualitative virtual target profiling^[3] was performed on a library of 5500 novel compounds designed by our organic chemistry group. This in silico screening identified a number of molecules bearing the chromene unit as potentially active on different receptor families known to play an important role in some of the pathways associated with cell proliferation and apoptosis in cancer cells.

In this work, a detailed discussion of the scope of the synthetic method for chromenes **1–3** (Scheme 1) will be presented.



Scheme 1. Novel synthetic chromene-based compounds that were biologically evaluated.

The anticancer potential of these compounds was evaluated in colorectal carcinoma cell line HCT116 by the MTT assay. IC_{50} values of the compounds were calculated, and the most active compounds were tested for their ability to induce cell cycle arrest and cell death by apoptosis.

Acknowledgements: we gratefully acknowledge financial support from the University of Minho and the FCT through the Portuguese NMR network (RNRMN), and the Project F-COMP-01-0124-FED-ER-022716 (ref. FCT PEst-C/UI0686/2011) FEDER-COMPETE and BPD grant awarded to Marta Costa (SFRH/BPD/79609/2011).

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Synthesis and Pharmacological Evaluation of New Chromene Scaffolds for Adenosine Receptors

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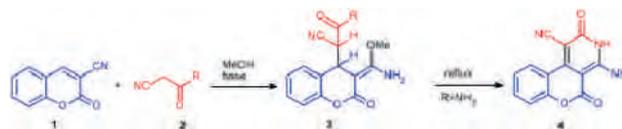
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Adenosine receptors are distributed throughout the body, regulating different cellular functions, and can be considered attractive targets for therapeutic agents.^[1,2] Different compounds proved to be active on these receptors, displaying pharmacological activity namely for the treatment of cardiovascular, inflammatory, or neurodegenerative diseases and cancer.^[1,2] The active molecules usually belong to the purine family, but compounds with the pyrazolo-triazolo-pyrimidine, dihydropyridine, and quinazoline-urea core unit were also identified as active.^[1] To our knowledge, the interaction of chromene derivatives with adenosine receptors was never reported.

The chromene scaffold is present in a variety of biologically active compounds and their synthesis has been widely explored in the literature.^[3] In this work, a one-pot procedure for the synthesis of novel chromene derivatives **3** and **4** is reported from the reaction of 2-oxo-2H-chromene-3-carbonitriles **1** and cyanoacetamides **2** (see scheme). These new scaffolds proved to be active at adenosine receptors, and several hits were identified in this study

with affinities in the submicromolar range. A detailed discussion of the synthetic method and affinities of the compounds will be presented.



Acknowledgements: We gratefully acknowledge the financial support from University of Minho and FCT through the Portuguese NMR network (RNRMN), the Project F-COMP-01-0124-FED-ER-022716 (ref. FCT PEst-C/UI0686/2011), and FEDER-COMPETE and BPD grants awarded to Marta Costa (SFRH/BPD/79609/2011) and Filipe Areias (SFRH/BPD/26106/2005).

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P482

Novel Steroid Inhibitors of Glucose-6-phosphate Dehydrogenase

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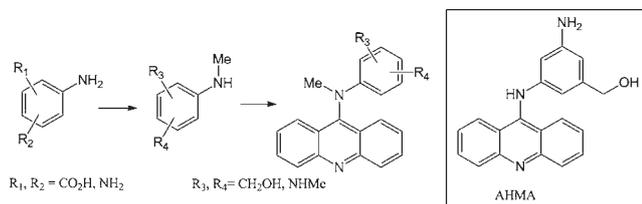
The rate-limiting enzyme of the pentose phosphate pathway (PPP) is glucose-6-phosphate dehydrogenase (G6PD) which catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconolactone and results in the production of NADPH. In addition to the production of biosynthetic precursors such as ribonucleotides, fatty acids and cholesterol, NADPH is important for maintaining glutathione in its reduced state and preventing cell stress arising from reactive oxygen species (ROS). Consequently, G6PD inhibitors may find therapeutic application by reducing the ability of tumour cells to manage ROS and thereby augment the effects of cancer radiotherapy or reduce the need for chemotherapy. We have identified a series of novel steroid G6PD inhibitors which modulate flux through the PPP in cells. This presentation will describe our latest results in this area.

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Facile Synthesis of Potential Antitumor Novel N(9)-Methylated AHMA Analogues

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The discovery of new compounds with antitumor activity has become one of the most important goals in medicinal chemistry. One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that are DNA intercalators. In previous studies on the development of potential 9-anilinoacridine intercalators, AHMA exhibited both in vitro and in vivo potent antitumor efficacy^[1] in mice bearing mammary and lung carcinomas. It was noticed that 9-anilinoacridines, in general, undergo a reversible amine exchange reaction at position nine under near-physiological conditions in water.^[2] This thermodynamically controlled reaction may have implications in understanding the mode of action of 9-anilinoacridines in vivo and in the future design of new drugs that will be based on this scaffold. These findings brought us to hypothesize that methyl substitution at amine nine of AHMA derivatives will influence such exchange reactions or hydrolysis of the aniline moiety, therefore shedding additional light on SAR of AHMA analogues. Here we report a new facile synthesis and biological activity of novel antitumor N(9)-AHMA derivatives.



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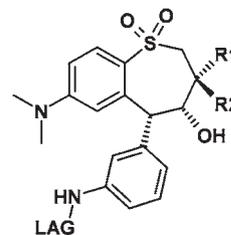
P484

Non-systemic IBAT Inhibitors to Modulate the Enterohepatic Circulation of Bile Salts: A New Approach for the Treatment of Hypercholesterolemia and Potentially in Diabetes

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Bile acids and their salts are of interest to the medicinal chemist due to their amphiphilic character and the more recently discovered regulatory and signaling functions in metabolism they exert via FXR and TGR5. Upon biosynthesis from cholesterol in the liver, bile acids are secreted with bile into the lumen of the small intestine to aid in the digestion and absorption of fat and fat-soluble vitamins. In the terminal ileum the bile acids are almost completely reabsorbed by a Na⁺-dependent transport system (IBAT or ASBT), they are then transported with portal blood to the liver and taken up into hepatocytes by a second Na⁺-bile acid cotransporter (LBAT), to be re-secreted into bile. Via FXR activation in the small intestine and the liver, bile acids decrease the activity of hepatic cholesterol-7 α -hydroxylase, the rate-limiting enzyme for the conversion of cholesterol into bile acids. This effect is reduced by interruption of the enterohepatic circulation of bile acids with IBAT inhibitors, leading to upregulation of hepatic LDL-receptors with a concomitant decrease of LDL-cholesterol. This general therapeutic principle to lower cholesterol levels has since been brought to clinical practice, along with polymeric bile acid sequestrants, while in recent years specific inhibitors of the intestinal IBAT/ASBT have been identified for this purpose. In addition, emerging evidence associates FXR activation by bile acids with alterations in triglyceride and glucose metabolism, and an effect of bile acids on GLP1 secretion from intestinal L-cells via TGR5 activation has been described, suggesting a contribution of (intestinal) bile acids to glucose homeostasis.



To exert a profound systemic effect, IBAT inhibitors do not need to be available systemically but can act from the luminal side in the small intestine. This offers the advantage to avoid some limitations of systemic hypolipidemic drugs, like statins, which are due to drug metabolism and drug–drug interactions in the liver. This also implies the need to follow other than standard pathways in compound optimization and drug development, and the concept of low-absorption drugs was established. In the presentation/poster, some mechanistic

and therapeutic aspects of the approach to interfere with intestinal bile acid absorption will be discussed, and an overview will be given on the molecular target, the discovery of selective low-absorption IBAT inhibitors, and respective optimization strategies.

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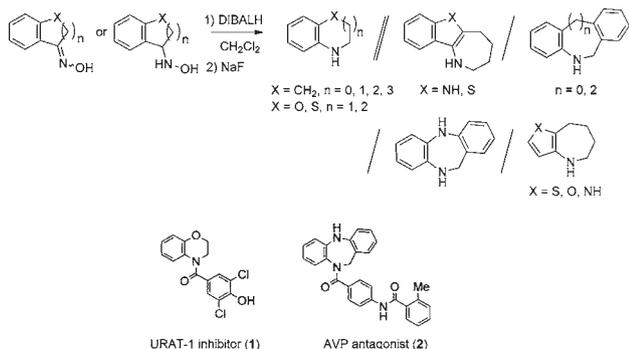
Novel Synthetic Approach towards the Core Structures of Medicines and Clinical Candidates. Regiospecific Rearrangement of Oxime or Hydroxylamine Using Diisobutylaluminum Hydride (DIBALH)

Hidetsura Cho, Yusuke Iwama, Nakako Mitsuhashi, Kenji Sugimoto, Kentaro Okano, Hidetoshi Tokuyama

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The human urate transporter-1 (URAT-1) inhibitor and the arginine vasopressin (AVP) antagonist were synthesized using the compounds obtained from the novel regiospecific rearrangement of heterocyclic ketoximes with DIBALH. This method will become useful for medicinal chemistry and process chemistry in the near future.

Development of novel synthetic methods for the unsubstituted basic skeletons of heterocycles is important from the viewpoint of both synthetic chemistry and medicinal chemistry. Synthesis of five- to eight-membered bicyclic or tricyclic fused heterocycles containing nitrogen neighboring an aromatic ring, such as hydrogenated benzazepine, benzoxazine, benzoxazepine, benzthiazine, benzthiazepine, dibenzodiazepine, dibenzoazocine, phenanthridine, and azepino[3,2-*b*]indole is particularly important because of the core structures of medicines or clinical candidates. We developed a novel synthetic method of oxime^[1] or hydroxylamine^[2] with DIBALH to afford the core compounds (see scheme). To demonstrate the usefulness of DIBALH reduction, we synthesized human URAT-1 inhibitor (**1**), AVP antagonist (**2**), and 17 beta-hydroxysteroid dehydrogenase type-3 inhibitor by using the resulting heterocycles. DIBALH is usable even in an industrial scale. Therefore, this methodology will serve both medicinal chemistry and process chemistry as a useful procedure in the near future.



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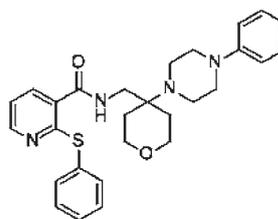
P490

Synthesis and Pharmacological Characterization of a Novel, Brain-Penetrating P2X7 Antagonist

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The discovery, synthesis and pre-clinical characterization of a novel, brain-penetrating P2X7 antagonist (compound **1**) will be discussed. The P2X7 receptor is a member of the purinergic family of ligand-gated ion channels that is abundantly expressed in glial cells in the CNS. ATP dependent gating of the P2X7 channel leads to activation of downstream signaling pathways and, among other pro-inflammatory processes, leads to IL-1 β secretion. The IL-1 β released from these glial cells is thought to play a role in initiating a neuroinflammatory cascade in the brain, which may contribute to various neuropsychiatric and neurodegenerative disorders. For these reasons we became interested in the identification of novel, brain-penetrating P2X7 antagonists that would be useful for characterization of the role of P2X7 in a variety of CNS disorders. In this presentation, we will report our progress towards that end and we will disclose the discovery of compound **1**, a novel, selective, high affinity P2X7 antagonist in human, rat and mouse cell lines ($K_i < 50$ nM in all three species). Compound **1** has reasonably good drug-like properties, has been extensively characterized pre-clinically, and has been found to effectively penetrate the rat brain (brain/plasma ratio 0.8). We will also discuss our efforts towards the development of a P2X7 rat brain receptor occupancy assay and will show that compound **1** effectively occupies the rat P2X7 receptor ($RO_{max} = 80\%$) as measured by ex vivo autoradiography, thus demonstrating that compound **1** is a useful tool for rat pharmacodynamic studies.



Compound **1**
 human PBMC $IC_{50} = 31$ nM
 human $K_i = 48$ nM
 rat $K_i = 10$ nM
 mouse $K_i = 34$ nM
 human whole blood $IC_{50} = 230$ nM

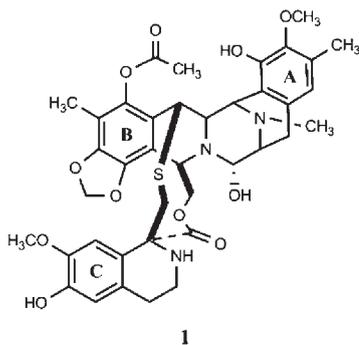
P491

Studies toward a Novel Total Synthesis of Ecteinascidin-743: Discovery of a New and Practical Synthesis of β -Lactams

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Ecteinascidin-743 (ET-743, also known as trabectedin; **1**) isolated from the Caribbean tunicate *Ecteinascidia turbinata*,^[1] is arguably the most potent cytotoxic known as indicated by its evaluation against the US National Cancer Institute's human in vitro cell line panel including melanoma, non-small-cell lung, ovarian, renal, prostate, and breast cancer, demonstrating potencies ranging from 1 μ M to 10 nM.^[2] In fact, the antiproliferative activity of ET-743 is greater than that of Taxol, camptothecin, adriamycin, mitomycin C, cisplatin, bleomycin, and etoposide by 1–3 orders of magnitude, propelling trabectedin (**1**) to become the first marine anticancer drug to be approved (October 2007) in the European Union (EU)^[3] as a first-line treatment for soft tissue sarcomas. The complexity of molecular architecture, the remarkable biological activities, and the restricted natural availability (1.0 g from about 1.0 ton of tunicate) have made **1** an exceedingly attractive synthetic target for total synthesis.^[4] Our studies toward the validation of key elements of our retrosynthetic analysis will be presented including the general and useful reaction of the title compound.



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P492

Phenolic Constituents from *Pulicaria undulata* Subsp. *undulata* and Their Antimicrobial Activity

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The aqueous methanol extract of *Pulicaria undulata* subsp. *undulata*, an Egyptian medicinal plant, was subjected to phytochemical investigation. One new natural isoflavone compound, 5,7,2',3'-tetrahydroxyl isoflavone-4'-O- β -D-glucopyranoside was isolated and identified, together with six known flavonols and one phenolic acid. Their structures were established through chemical and spectral analysis.

The extract and its constituents were examined for antibacterial and antifungal activity in vitro using the cell-diffusion method. The methanol extract, together with the isolated compounds, showed activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*.

P494

Surface Components Contribute to the Virulence in *Klebsiella pneumoniae* Causing Pyogenic Liver Abscess

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Klebsiella pneumoniae is one of the organisms most commonly isolated from pyogenic liver abscess (PLA) in Asian populations for two decades. Pyogenic liver abscess (PLA) is associated with significant morbidity and mortality, because it develops into sepsis and bacteremia. There are around three billions people, almost half of the world population, in the Asian region. For prevention of PLA, *K. pneumoniae* is significant critical right now. In clinic medicine, to develop a vaccine targeted by surface structure of PLA *K. pneumoniae* may reduce the liver abscess formation in those who have diabetes or in weak immune function.

Both capsular polysaccharide (CPS) and lipopolysaccharide (LPS) of clinic PLA *K. pneumoniae* isolates were studied. PLA *K. pneumoniae* CPS induces secretion of TNF- α and IL-6 by macrophages through TLR4 and regulated by the TLR4/ROS/PKC- δ /NF- κ B, TLR4/PI3-kinase/AKT/NF- κ B, and TLR4/MAPK signaling pathways. Moreover, CPS induces caspase-1-dependent IL-1 β secretion in the presence of ATP. Depletion of NLRP3 and its interacting adaptor protein ASC result in decreased caspase-1 activation and IL-1 β secretion, but no decrease

in TNF- α and IL-6 secretion is observed in response to CPS and ATP. CPS induced NLRP3 inflammasome and IL-1 β precursor (proIL-1 β) expression through reactive oxygen species (ROS)-, ERK1/2-, and p38-associated pathways. Mitochondrial ROS and mitochondrial membrane permeability transition were found to be important for NLRP3 inflammasome activation in response to both CPS and ATP. The anti-CPS monoclonal antibodies protected mice from magA⁺ *K. pneumoniae*-induced liver abscess formation and lethality. This indicates that the K1 epitope is a promising target for vaccine development.

Serological analysis of *K. pneumoniae* clinical isolates demonstrated that the O1 serotype was more prevalent in PLA strains than that in non-tissue-invasive strains (38/42 vs 9/32, $p < 0.0001$). O1 serotype isolates had a higher frequency of serum resistance, and mutation of the O1 antigen changed serum resistance in *K. pneumoniae*. Our findings indicate that O1 antigen contributes to virulence by conveying resistance to serum killing, promoting bacterial dissemination to and colonization of internal organs after the onset of bacteremia, and could be a useful vaccine candidate against infection by PLA *K. pneumoniae*.

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P495

Imidazolium Arylacetates: Ionic Liquids for Drug Release

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 M^a José García-Celma,^[b] Ferran Roig^[b]

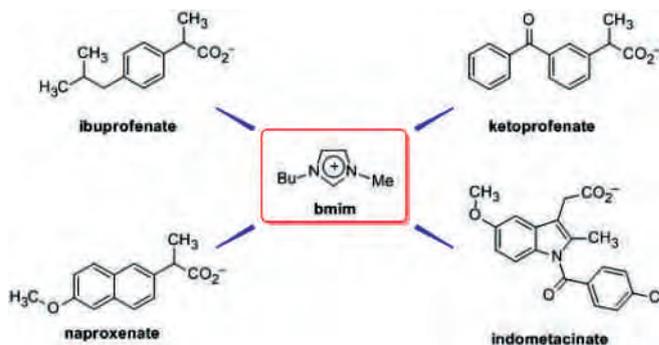
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Besides their recognized value as an alternative to conventional solvents, ionic liquids (ILs) are becoming increasingly useful in a widening the range of fields in chemistry leaning toward biology. ILs make a unique architectural platform on which the properties of both cation and anion can be independently modified, providing tunability in the design of new functional materials as well as pharmaceutical and biological ingredients. In this way, their use enables to modulate the properties of active pharmaceutical ingredients (APIs) with novel performance enhancement and delivery options.

As a part of our ongoing research, we recently reported the anion exchange procedure in non-aqueous media as a simple method of choice to swap the halide ion of ILs for a broad range of anions, including ibuprofenate.^[1] In order to extend our protocol to anti-inflammatory arylacetic acids, we herein report the preparation of several [bmim][R-CO₂] following AER (A⁻ form) method from selected

examples of nonsteroidal anti-inflammatory drugs (NSAIDs). In addition, the study of the release from hyaluronan-based hydrogels as drug delivery system was carried out, considering that the high biocompatibility of this natural polysaccharide provides a good candidate for biomedical and pharmaceutical use.



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P496

Synthesis and Biological Evaluation of Benzoxazines and Quinazoline-3-oxides

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Benzoxazines and their analogues are a significant class of heterocycles involved in various biological properties such as inhibitory activity towards human leukocyte elastase and Clr serine protease enzymes. Numerous benzoxazine analogues were evolved as DNA binding antitumor agents and also act as progesterone receptor modulators. Several polymeric benzoxazines were explored as heat resistant and electronic materials. 4-Arylidene-2-aryl-4H-benzo[d]-[1,3]oxazines are synthesized with high stereoselectivity and regioselectivities from 2-alkynylbenzamides in the presence of catalytic amount of I₂. In the reaction mechanism, iodine plays a key role in two different aspects as a catalyst, such as to activate the alkyne with the iodonium donor which triggers the cascade, and then as a proper acid source to barrage catalyst recovery. The benzoxazines have been exploited as potential substrates for the synthesis of quinazoline-3-oxide derivatives directly in one step. Some compounds were found to show photodynamic therapy (PDT) applications against the melanoma as well oral cancer cell lines.

P497

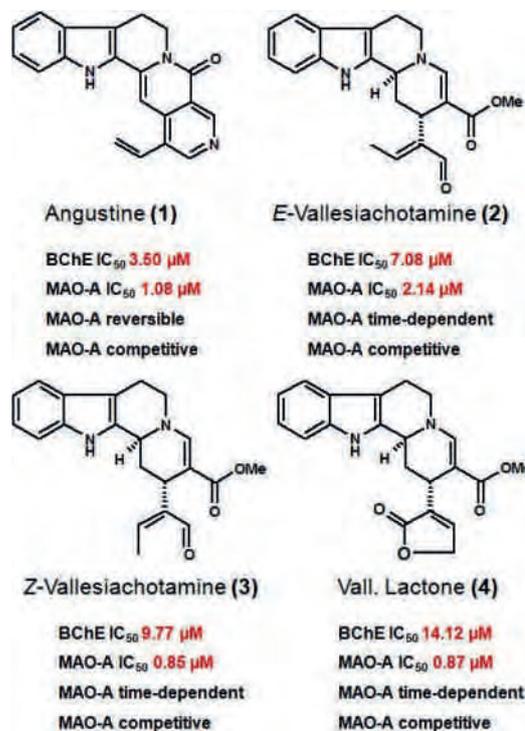
Alkaloids from *Psychotria* as Multifunctional Cholinesterases and Monoamine Oxidases Inhibitors

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Psychotria L. is a taxonomically complex genus whose neotropical species (subg. *Heteropsychotria*) are characterized by the presence of monoterpene indole alkaloids (MIAs) possessing biological and pharmacological properties on the CNS, mainly related with the serotonergic and glutamatergic transmission.^[1,2] Therefore, considering the presence of MIAs with β -carboline (β Cs) and tetrahydro- β -carboline (TH β Cs) nuclei in *Heteropsychotria* and the biological activities assigned to the substances belonging to this class, it becomes relevant to investigate the activity of these compounds on enzymatic targets related with neurodegenerative diseases. In the present study, 11 *Psychotria* alkaloids were evaluated for their inhibitory activity on acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and human monoamine oxidases (MAO-A and -B). Angustine (**1**), *E/Z*-vallesiachotamine (**2**, **3**), and vallesiachotamine lactone (**4**) were able to inhibit more than 50% of BChE and MAO-A in concentrations of 10 μ M. These compounds displayed IC₅₀ values ranging between 3–15 μ M on BChE and 0.8–2.5 μ M on MAO-A (see figure).

The time-dependence and kinetic studies on MAO-A indicated that **1** acts as a reversible and competitive inhibitor, such as other β Cs and TH β Cs. On the other hand, alkaloids **2**, **3** and **4** seem to inhibit MAO-A in a time-dependent way. In silico molecular interaction studies structurally support such data. Taken together, our findings revealed molecular details of BChE and MAO-A inhibition by MIAs from *Psychotria*, suggesting that these compounds could consist of scaffolds for multifunctional ligands for both enzymes, which are involved in the pathophysiology of Alzheimer's disease.



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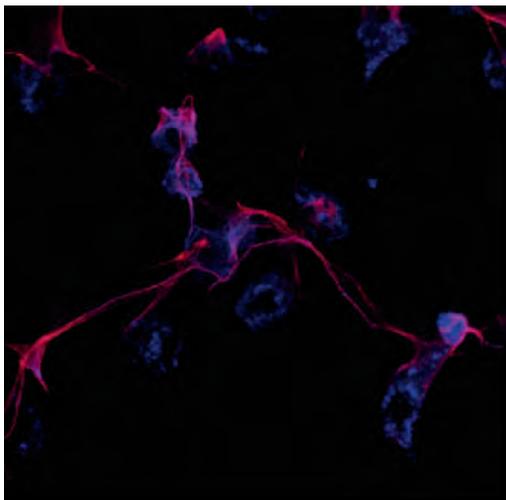
Identification of Novel Inhibitors of Neutrophil Extracellular Traps (NETs)

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Polymorphonuclear neutrophils are short-lived leukocytes that migrate to infected sites under the acute immune response where they phagocytose, and degranulate to kill microbes.^[1] Additionally, neutrophils were found to form neutrophil extracellular traps (NETs).^[2–3] In this process, known as NETosis, the nuclear lobules disappear, the chromatin expands and the DNA is expelled and is decorated with

antimicrobial proteins. The signalling mechanisms leading to the formation of NETs are poorly understood.^[4] In addition, neutrophils are terminal cells and are not suitable for conventional analytical techniques, which complicates their study using genetic or other biological approaches. Here, we envisage a chemical biology approach to investigate the signalling processes regulating NET formation. The focus of this project is to identify the molecular targets involved in the NET formation process by using chemical inhibitors that may block this process at different stages. At the same time, this approach may lead to the identification of novel therapeutic entities. To achieve this, a screen was first developed using isolated human neutrophils and a commercial library was screened. Here, the inhibition of induced NET formation was analyzed using nuclear morphology and cell viability as readouts in a single protocol. As a result of this screen, we could show that the Raf/MEK/ERK pathway is involved in NET formation through activation of NADPH oxidase and up-regulation of anti-apoptotic proteins.^[5] In addition, the screening of a chemical library generated in the group enabled the identification of a novel compound class inhibitors of NET formation. Further investigations to identify the molecular target of these inhibitors are on-going.



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P499

Evaluation of Salicylic Amides as S100A9 Binders Inhibiting S100A9–RAGE and S100A9–TLR4 Interactions

Ulf Wellmar, Per Björk, Camilla Gummesson, Martin Stenström, David Liberg, Tomas Leanderson

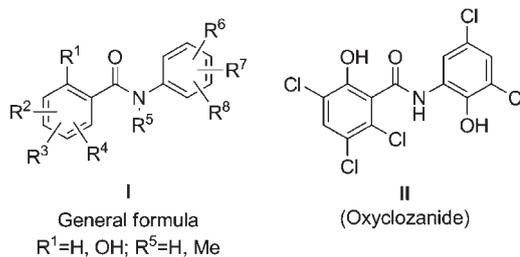
Active Biotech AB, PO Box 724, 220 07 Lund, Sweden

S100A9 has roles in both inflammation^[1] and cancer^[2] and has previously been demonstrated to be a target for Active Biotech's quinoline compounds.^[3] To investigate the general feasibility of S100A9 as a drug target, we started to explore other classes of compounds as potential inhibitors of this specific target.

Early on in this activity, we identified salicylic amides to be a promising starting point for further development, and smaller libraries of compounds based on the general formulae I were designed, synthesized and tested for binding to S100A9. The testing was performed using surface plasmon resonance technology to detect inhibition of S100A9 binding to RAGE and TLR4.

After several rounds of SAR-driven synthesis, optimized structures started to emerge. It was concluded that benzoic amides ($R^1=H$) in general are less potent than corresponding salicylic amides ($R^1=OH$) and that *N*-substitution ($R^5=Me$) renders less active compounds. Larger substituents on either phenyl are accepted but the most potent compounds have the common feature of a high degree of halogenation without added bulk, one example being the known anthelmintic drug, oxiclozanide (II).

The most potent compounds were tested for cytotoxic and anti-proliferative effects. Oxiclozanide was shown to be the most favorable compound in vitro, was tolerated in mice and had acceptable pharmacokinetic properties. Finally, in vivo testing showed effects in two S100A9 dependent models. Thus, we conclude that development of small molecule S100A9–RAGE/TLR4 inhibitors is feasible and renders biologically active compounds.



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P500

Curcumin—Tearing Down the Wall between Spice and Drug

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Curcumin is a yellow spice that naturally occurs in tumeric (*Curcuma longa*) plants. It is not only utilized in Indian cuisine, but also in traditional Indian medicine to treat a number of disorders.^[1,2] This simple, yet highly functionalized natural product has been extensively investigated for its medicinal properties for the last 5 decades, and more recent studies have shown that curcumin analogues are inhibitors of pSTAT3 activation, a potential target for therapeutic intervention in head and neck squamous cell carcinoma (HNSCC, Figure 1a).^[3,4]

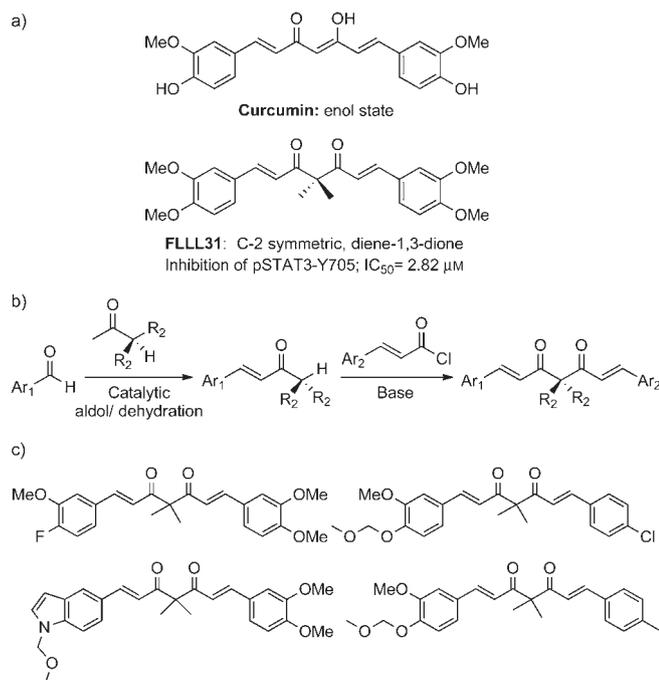


Figure 1. a) Curcumin and FLLL31, an inhibitor of pSTAT3 in HNSCC. b) Facile method for curcumin analogue “Matyrix” synthesis. c) Examples of curcumin analogues synthesized with pSTAT3 inhibition.

Despite a number of published reports describing curcumin analogues with pSTAT3 inhibition, no curcumin analogue has been disclosed with the drug-like properties that would make it an acceptable candidate for drug development.^[5,6] This shortcoming is due to the limited methodology available to meet the synthetic challenge of constructing the diene-1,3-dione while allowing for an exploration of comprehensive structure–activity relationships (SAR). A medicinal chemistry effort that could exploit curcumin as viable drug candidate for further clinical development is clearly needed. Recently, in an attempt to synthesize a known curcumin analogue with pSTAT3 inhibition, FLLL31, our group took advantage of a retrosynthetic ap-

proach that has been largely overlooked in the literature. From the outset, the goal of this synthetic approach was to allow for a broad SAR investigation (Figure 1b).

Our methodology toward curcumin analogues fully exploits this unique ethnopharmacologic compound via a matrix parallel synthesis strategy that takes advantage of a convergent building block assembly. In addition to a discussion of the scope of this transformation, we will describe the properties of a diverse set of analogues, and their respective biological activity as inhibitors of pSTAT3 (IC₅₀) in HNSCC (Figure 1c).

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P501

Synthesis and Docking Studies of Novel Anti-HCV Benzimidazoles

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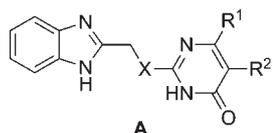
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Different benzimidazole derivatives have been studied as hepatitis C virus inhibitors.^[1] In this study, synthesis and docking studies of a series of new benzimidazole derivatives **A** linked to substituted pyrimidines either through methylthio linkage or by its bioisosteric methylamino bridge were carried out. All the synthesized compounds were evaluated for their hepatitis C virus viral replication inhibitory activities. Compounds **4d,e,h** showed to be more potent than VX-950 (IC₉₀ **4d**=0.321, **4e**=0.345, **4h**=0.432, VX-950=0.45 μM) and comparable to JTK109 (IC₅₀(NS5B:1b)=0.017 μM). The binding

mechanisms and interactions of the compounds were predicted with docking studies using the molecular operating environment (MOE). The inhibitors had best docking scores and interactions with active site residues in the polymerase GTP binding site that connects the thumb and palm subdomains. The active compounds had comparable docking scores and binding mechanism to JTK109.



X=S, N
R¹=phenyl, substituted phenyl
R²=CN, COOH

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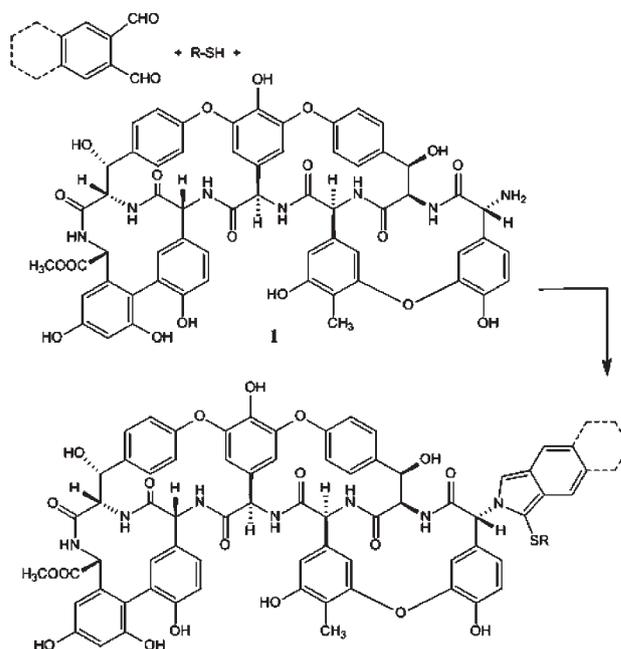
P502

Synthesis of Fluorescent Derivatives of Glycopeptide Antibiotics with Remarkable Antibacterial and Antiviral Activities

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Glycopeptide antibiotics vancomycin and teicoplanin are used for treating serious Gram-positive bacterial infections that are resistant to other antibiotics.^[1] The emergence and spreading of glycopeptide-resistant enterococci (GRE) and glycopeptide intermediate resistant *Staphylococcus aureus* (GISA) lead to an urgent need for new antibiotics active against resistant bacteria and initiated an intensive research in this field in the past 20 years.^[2] Ristocetin A is a glycopeptide-type antibiotic produced by *Nocardia lurida*. This molecule consists of six sugar moieties attached to the aglycon (**1**). In spite of its good antibacterial activity against Gram-positive strains this antibiotic has not been used in therapy due to its ability to cause aggregation of blood platelets. In the past few years, we have synthesized a series of new aglycoristocetin derivatives with not only high antibacterial activity but in several cases with robust anti-influenza virus properties.^[3] Here we report the synthesis and physicochemical and biological evaluation of a series of aglycoristocetin and teichoplanin pseudo-aglycon isoindole derivatives. It was found to be a successful effort for synthesizing fluorescent peptidoglycan derivatives with considerable antibacterial and anti-influenza activities.



In case of the isoindole conjugates of teichoplanin pseudo-aglycon isoindole, antibacterial tests confirmed minimum inhibitory concentrations in the low ng/mL range for several fairly perilous resistant strains. One possible explanation for these remarkable biological properties could be the special case of multivalence achieved in the form of aggregation, which was demonstrated by light scattering studies.

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P503

From a Potent 5-HT_{2C} Inhibitor to a Fast-Killing Antimalarial Scaffold in 26 Compounds

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From the 13,533 chemical structures published by GlaxoSmithKline in 2010, we identified 47 quality starting points for lead optimization. One of the most promising hits was the TCMDC-139046, a molecule presenting an indoline core, which is well known for its anxiolytic properties by interacting with serotonin antagonist receptors 5-HT₂. The inhibition of this target will complicate the clinical development of these compounds as antimalarials. Herein, we present the antimalarial profile of this series and our efforts to avoid interaction with this receptor, while maintaining a good antiparasitic potency. By using a double-divergent structure-activity relationship analysis, we have obtained a novel lead compound harboring an indoline core.

P504

Effect of Electron Density on the Indole Ring of Tryptophan Derivatives on Transcriptional Regulation of Indoleamine 2,3-Dioxygenase (IDO)

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Indoleamine 2,3-dioxygenase (IDO) is the initial and rate-limiting enzyme in the metabolism of the essential amino acid L-tryptophan along the kynurenine pathway in mammalian cell. The enzyme is induced predominantly in many cell lines by stimulation with interferon (IFN)- γ . By depleting tryptophan locally, IDO blocks the proliferation of T-lymphocytes, which are sensitive to tryptophan shortage.^[1] IDO expression could suppress immune responses by blocking T-lymphocyte proliferation locally. A large body of evidence has been accumulating for its immunosuppressive and tumoural escape roles and its applicability as a therapeutic target.

We previously have reported that L-tryptophan, as a substrate of IDO, stimulated IFN- γ -inducible IDO expression at the transcriptional level in human epidermoid carcinoma cell line A431.^[2] In this study, we examined the effects of other amino acids and tryptophan analogues on the IFN- γ -inducible IDO expression. 6-Nitro-L-tryptophan increased IFN- γ -inducible IDO expression by threefold, but in the presence of 5-hydroxy-L-tryptophan, no effect stimulating IDO expression was seen. In the presence of L-methionine and L-phenylalanine, other essential amino acids, no effect stimulating IDO expression was detected.

We also performed the ab initio molecular orbital calculation for these tryptophan analogues, in order to elucidate the mechanism of the IDO expression. The geometrical optimization was carried out and the molecular electrostatic potential was calculated for each analogue. The electrostatic potential maps enable us to visualize the charge distributions of molecules and charge related properties of molecules. We found that there was a strong positive correlation between the charge distribution on the molecular surface and IFN- γ -inducible IDO expression level using A431 cells. A high correlation coefficient ($r^2=0.887$) was obtained.

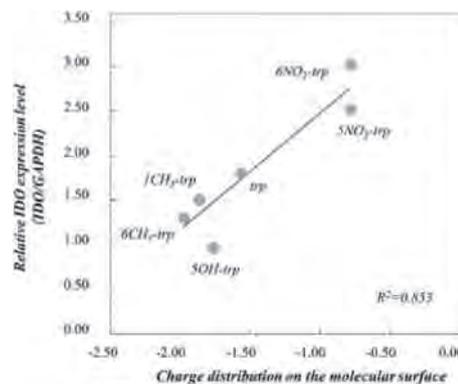


Figure 1. Correlation of relative IDO expression level and charge distribution on the molecular surface

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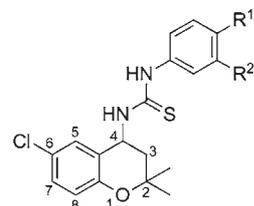
***N*-(6-Alkoxy-carbonylamino-3,4-dihydro-2*H*-1-benzopyran-4-yl)-*N'*-arylureas Display In Vitro Anticancer Activity on Apoptosis-Resistant Glioblastoma Cells**

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Philippe Gailly,^[e] Bernard Rogister,^[b] Robert Kiss^[d]

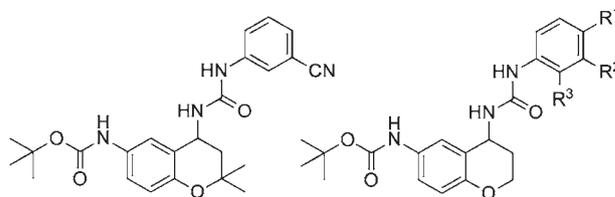
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In the search of new pharmacological agents intended for the treatment of glioblastoma, 3,4-dihydro-2*H*-1-benzopyrans, diversely substituted at the 4-position with arylurea or arylthiourea side chains, were screened for their antiproliferative activity on several apoptosis-resistant glioma cell lines (Hs683, U373 and T98G). A preliminary in vitro screening of such activity by means of a colorimetric MTT test allowed us to identify active compounds in the micromolar range, such as BPDZ 566 and BPDZ 569, characterized by the presence of a nitrophenylthiourea moiety at the 4-position and a chlorine atom at the 6-position. Moreover, tests on normal glial cells from mice revealed that the presence of an arylurea chain instead of an arylthiourea chain was responsible for a better selectivity for the tumor versus normal cells, in spite of a lower antiproliferative activity. As a result, a new in vitro screening on the glioma cell lines was performed with new series of arylurea-substituted benzopyrans bearing an alkoxy-carbonylamino ('carbamate') function at the 6-position. The best results were obtained with the *tert*-butoxycarbonylamino-substituted derivative, BPDZ 711, which was able to reduce by 50% the proliferation of the U373 cancer cells at the low concentration of 2 μ M and which proved to be selective for the tumor versus normal glial cells (selectivity index =10). Surprisingly, BPDZ 711 blocked the cell cycle in the S phase instead of the G0/G1 phase like BPDZ 566 and BPDZ 569.

The critical role of the presence of two methyl groups at the 2-position was also studied on new examples of 6-*tert*-butoxycarbonylamino-substituted benzopyrans bearing an arylurea moiety at the 4-position, since it was recently observed that such a structural modification provided active compounds on glioma cells.



BPDZ 566: R¹=NO₂; R²=H
BPDZ 569: R¹=H; R²=NO₂



BPDZ 711

BPDZ 752-757
R¹, R², R³=H, Cl, CN

New pharmacomodulations, such as the synthesis of thiocroman analogues of BPDZ 711, will be explored in the near future in order to complete our knowledge of the structure–activity relationships.

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Analgesic, NO Donor and Fetal Hemoglobin Inducer Properties of Furoxanyl Derivatives Useful to Treat Sick Cell Disease Symptoms

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Introduction: Sick cell disease (SCD) is a hematological disease characterized by punctual mutation of β Glu6 in Hb to β Val6 in HbS. The disease has an important inflammatory component. The increased pro-inflammatory cytokines promote cell adhesion to vascular endothelium contributing to vaso-occlusion process—main responsible for pain. Nowadays, hydroxyurea (HU) is the only drug approved by US Food and Drug Administration (FDA) to treat SCD. It has been described that after metabolism, HU is converted to nitric oxide which is responsible for numerous HU benefits, including the induction of γ -globin gene expression, vasodilatation and the inhibition of platelet aggregation. In addition, several reports

demonstrated that thalidomide and some derivatives (pomalidomide and lenalidomide) are able to induce γ -globin gene expression and HbF synthesis.^[1] Furthermore, this compound demonstrated analgesic activity by inhibition of tumor necrosis factor alpha (TNF α). Our rational drug design used molecular hybridization between thalidomide and NO donor subunits, represented by furoxanyl derivatives. The aim of this study was to evaluate the effects of novel hybrid compounds (I–VIII) on analgesic activity, NO donor ability and γ -globin gene expression.

Methodology: 1. *Antinociceptive activity.* Analgesic activity was determined in vivo with the acetic-acid-induced (0.6%, 0.1 mL/10 g) abdominal constriction test in mice. Swiss mice of both sexes (18–23 g) were used. The compounds were administered orally (100 μ mol/kg) as a suspension in 5% arabic gum in saline (vehicle). Dypirone (100 μ mol/kg) was used as the standard drug. Acetic acid solution was administered i.p. 1 h after the administration of the compounds. Ten minutes after the i.p. acetic acid injection, the number of constrictions per animal was recorded for 20 min. The control animals received an equal volume of vehicle. Antinociceptive activity was expressed as percentage inhibition of the constrictions compared with those in the vehicle-treated control group. The data were analyzed statistically with Student's t test at a significance level of $P < 0.05$. 2. *Detection of nitrite.* A solution of the appropriate compound (20 μ L) in DMSO was added to 2 mL of a mixture of 50 mM phosphate buffer (pH 7.4) and methanol (1:1, v/v), containing L-cysteine (5 mM). The final concentration of compound was 10–4 M. After 1 h at 37 $^{\circ}$ C, 1 mL of the reaction mixture was treated with Griess reagent (250 μ L). After 10 min at RT, the absorbance was measured at 540 nm using a spectrophotometer. Standard sodium nitrite solutions (10–80 nmol/mL) were used to construct the calibration curve. The yields of nitrite are expressed as % NO $^{2-}$ (mol/mol). 3. *γ -Globin gene expression.* Human K562 cells were maintained in DMEM with 10% FBS, Pen/Strep, in humidified air (5% CO $_2$, 37 $^{\circ}$ C). Cells (1x10 7 cells/100 mL) were incubated with compounds at different concentrations (5, 30, 60, and 100 μ M) for 24, 48, 72 and 96 h. γ -Globin gene expression was analyzed by qRT-PCR and quantified using the Gnorm program. Results are expressed as arbitrary units.

Results: 1. *Antinociceptive activity.* Compounds III and IV were the most active antinociceptive compounds reducing the acetic-acid-induced abdominal constrictions higher than control dypirone which inhibited 34%. Compounds III and IV inhibited the abdominal constrictions by 43% and 38%, respectively. 2. *Detection of nitrite.* The quantification of the nitrite produced resulted from the oxidative reaction of NO, oxygen and water. Compounds I–VIII were capable to induce nitrite formation at different concentrations between 9.5% and 28%. Isosorbide dinitrate (DNS), used as the control, induced 11.7% nitrite formation. 3. *γ -Globin gene expression.* Compound III was selected to evaluate γ -globin expression. This compound demonstrated the highest levels of γ -globin induction at 5 and 30 μ M. Compound III achieved maximal γ -globin induction as early as 48 h after treatment ([48h; 5 μ M]: 1.88 \pm 0.06 AU; control 0.78 \pm 0.1 AU; $P < 0.05$).

Conclusions: The molecular hybridization between thalidomide derivatives and NO donors seems to be an important strategy to discover new compounds to treat SCD symptoms. These new com-

pounds presented analgesic activity, NO donor ability and γ -globin inducer properties. Compound III is a new drug candidate to treat SCD symptoms.

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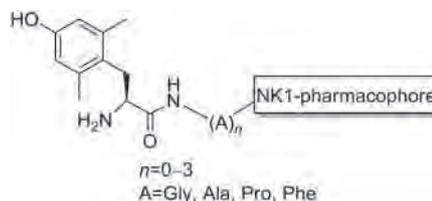
Dual Pharmacophore Ligands for Novel Pain Treatments

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In order to target different mechanisms involved in pain, dual pharmacophore ligands were developed. Compounds were designed using a peptidic pharmacophore with known opioid receptor activity combined with a non-peptidic pharmacophore interacting with the NK1 receptor. Some of these compounds potentially have less addictive properties than the parent morphine-like opiates. Further, the known anti-emetic activity of the NK1 antagonists such as Aprepitant would be beneficial, as it counteracts vomiting, one of the main issues of oral use of opiates.



Opioid peptide sequences were successfully linked with different NK1-scaffolds. Depending on the NK1 antagonist/opioid peptide combination we identified compounds that range from selective and potent mu- or delta-agonists to balanced mu agonists/NK1 antagonists; delta agonists/NK1 antagonists and mu/delta agonists/NK1 antagonists. The synthesis, SAR and pharmacological profile will be discussed.

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Synthesis of a Fullerene Derivative Containing N-Acetylneuraminic Acid

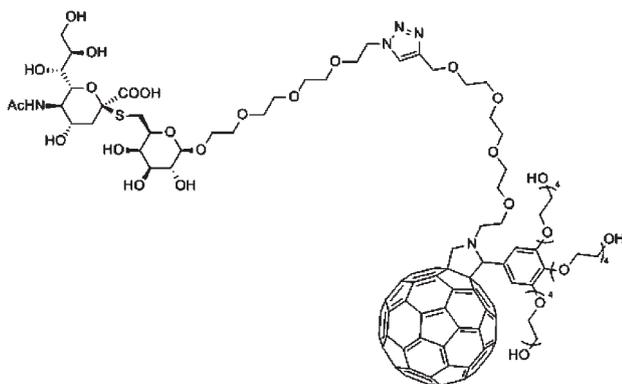
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Previously, we reported on a fullerene derivative of teicoplanin pseudo-aglycone displaying moderate antibacterial activity against certain types of bacteria, as well as antiviral activity against influenza A viruses.^[1]

Hemagglutinin is a protein of the influenza virus, and it is responsible for the attachment of the virus to the host cells via binding to its neuraminic acid-containing surface glycoproteins.

Our aim was the synthesis of a multivalent ligand that is expected to be resistant to neuraminidase enzyme and is able to attach to the hemagglutinin of the virus. We chose the extremely lipophilic fullerene molecule as a carrier of one of the terminal sialogalactoses of influenza virus-binding cell glycoproteins. For the linking of the neuraminyl-galactose thiodisaccharide to the tetraethylene-glycol-containing pyrrolidino-fullerene derivative, the copper-catalyzed alkyne-azide 1,3-dipolar cycloaddition reaction, the Click reaction, was chosen. These conjugates form aggregates in aqueous media resulting in N-acetylneuraminic acid clusters.



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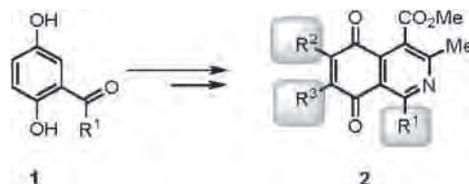
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Cytotoxic Activity of New Aminoisoquinoline-5,8-quinone Derivatives

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The molecular framework of several naturally occurring antitumoral agents contains an aminoquinonoid moiety as the key structural component (e.g., streptonigrin, mitomycin C, cribrostratin 3 and caulibugulones A–C). This structural array has stimulated the synthesis of novel lead compounds that exhibited significant cytotoxicity on human cancer cell lines. As part of a research programme on anti-tumor evaluation of isoquinoline-containing quinones,^[1] we have previously reported evidences on the influence of arylamino and methyl substituents on the in vitro cytotoxic activity on cancer cell lines.^[2] In order to better understand the structural determinants for potent and more selective antitumor compounds, we have synthesized a series of novel 4-methoxycarbonyl-3-methylisoquinoline-5,8-quinone derivatives type **2** (R^1 : H, Me, aryl and R^2/R^3 : alkylamino/H; arylamino/H; alkylamino/Cl, Br; arylamino/Br). The members of the series were prepared by substitution reactions of isoquinoline-quinones which were obtained through a highly efficient one-pot procedure from acylhydroquinones **1** and methyl aminocrotonate. The in vitro evaluation of the members of this series against human lung fibroblasts, gastric adenocarcinoma, lung cancer, and bladder carcinoma cell lines indicate that the nature and location of the nitrogen substituents were essential for a high cytotoxic activity. We have also observed that insertion of methyl, aryl and halogen groups into the aminoisoquinolinquinone chromophore induce significant effects on the cytotoxic effects and on the selectivity index.



The synthetic procedures applied for the preparation of the new isoquinolinequinones and the results of the biological evaluation towards the cancer cell lines will be presented and discussed.

Acknowledgments: This research was supported by FONDECYT (grant no. 3120023; 1060591) and the Pontificia Universidad Católica de Chile (V.D.; POSTDOC/N°1/2010).

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P510

Discovery of a Potent and Selective PIM1 Inhibitor by Rational Compound Evolution

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PIM1 is a constitutively active serine/threonine kinase, and its expression is increased in hematopoietic and prostate cancer patients. PIM1-transgenic mice show increased susceptibility to tumorigenesis, and PIM1-deficient mice do not display any overt abnormalities. Therefore, PIM1 is emerging as an attractive therapeutic target. To discover a novel type of PIM1 inhibitor, we rationally evolved a screening hit compound, which we have reported previously.^[1] We carried out the optimization study based on the structural information and confirmed that introduced chemical groups participate in interactions as designed by solving the structure of the representative compounds. In addition, we monitored the effectiveness of our medicinal chemistry effort by calculating the quality indices such as ligand efficiency (LE) and ligand lipophilicity efficiency (LLE) to control ADMET properties. As a result, we discovered a novel type of potent, selective, and metabolically stable PIM1 inhibitor that is a promising lead compound for further optimization.^[2]

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Metabolomics and Medicinal Chemistry, toward the Discovery of New Therapeutic Targets: Application to Age-Related Macular Degeneration (AMD)

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Metabolomics is one of the most recent technologies in the world of Omics sciences. It aims to study metabolome, which is composed of low-molecular-weight organic molecules (called metabolites) of a cell, an organism or a biological system. This approach gives rise to a growing number of applications in many areas, such as biomarker discovery, clinical studies, drug efficacy and toxicity evaluation, diagnostic tool, quality control. One of the most interesting features of metabolomics is its capability to extract biochemical information reflecting biological events and then to be a powerful tool in the knowledge of the aetiology of some pathology. Indeed, it is clear that every disease could alter more or less drastically the metabolic profile of the patients. Then a metabolomics approach could highlight the biochemical pathways affected and could allow the identification of new putative therapeutic strategy or targets that could be useful in a new drug discovery strategy. As proteomics, the metabolomics approach represents a new and powerful tool for medicinal chemistry.

Age-related macular degeneration (AMD) is a leading cause of vision loss in the western world among people aged 50 or older. 90% of all vision loss due to AMD result from the exudative form, which is characterized by choroidal neovascularization (CNV). Age-related changes that induce pathologic CNV are incompletely understood. A successful application of anti-VEGF approaches in the clinic is obviously a turning point in AMD treatment. Nevertheless, despite such important advances, critical issues remain to be addressed. To better understand the aetiology of this pathology, we have used and improved a murine model of laser-induced choroidal neovascularization. As none is known about the metabolic changes in patients with AMD, we decided to apply a ¹H NMR metabolomics approach on AMD patients and on the mice CNV experimental model. For this purpose, sera from healthy and AMD patients, induced and non-induced mice have been collected and the metabolic profiles of these samples were determined by ¹H NMR. After post-processing treatments, the different spectra were analyzed by statistical discriminant methodologies (PCA, ICA, PLS-DA).

This approach allows the differentiation between healthy and AMD patients and between laser-induced mice and the control mice group. Interestingly, the same discriminating spectral zones have been identified in human and mice model, leading to the emergence of different putative biomarkers and to the validation of the CNV model for an experimental study of AMD. Some of these metabolites (i.e., lactate and low-density lipoproteins) appear to be clearly involved in the development of AMD. The modulation of their plasma concentration by treatment of the animals with synthetic compounds significantly decrease the impact of laser-induced CNV, opening new treatment opportunities in human. So, the metabolomics approach has highlighted some biochemical pathways implied in AMD and led to a better comprehension of its aetiology. Moreover, these results have given rise to a new putative therapeutic strategy to reduce or suppress AMD impact.

P512

The Identification, Design and Synthesis of Inhibitors of the Thiolperoxidase (TpX) from *Mycobacterium tuberculosis* (Mtb)

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According to the World Health Organisation (WHO), tuberculosis is still today one of humankind's scourges with 8.8 million incident cases globally and with increasing cases of MDR-TB and XDR-TB, which explain the urgent need for new drugs against *Mycobacterium tuberculosis* (Mtb).

Mtb is an intercellular organism that lives and survives in macrophages by detoxifying reactive oxygen and nitrogen species produced by those same macrophages. From all the enzymes that form Mtb detoxifying system, the thioredoxin peroxidase (TpX) has been shown to be essential to deal with oxidative and nitrosative stress.^[1]

TpX is an atypical thiolperoxidase with NADPH-linked activity. Its peroxidatic cysteine, Cys60, is oxidized to cysteine sulfenic acid and the resolving cysteine, Cys93, attacks the sulfenic cysteine to form a disulfide bond that is later catalyzed by a cell-specific oxirreductase. The enzyme catalytic site is formed by an amino acids triad: Thr57, Cys60 and Arg130.^[2]

For our work we used a structure-based modelling approach with the help of Discovery Studio software. Based on a number of 3 to 5 different mapped features, varied pharmacophores were designed for two experiments: one within the amino acids triad and a second within the Cys60; allowing us to screen databases of compounds. The database screen results, in total a few thousands of compounds (56,969), were filtered using the Lipinski filter in order to get the most "druggable" molecules. On GOLD software, the filtered molecules were docked and then ranked according to a ratio of GoldScore divided by the molecule total number of atoms giving us the compounds with better in silico binding activity. The top 100 compounds

ranked were clustered by their characteristic scaffolds and functional groups, and lower scored analogues were removed to get the most varied number of molecules.

The compounds found using this drug discovery approach will be screened for in vitro activity using a biochemical assay.

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P513

Synthesis of Natural-Like Hydroxylated Biphenyl Chalcones and Aurones as Potential Bioactive Agents

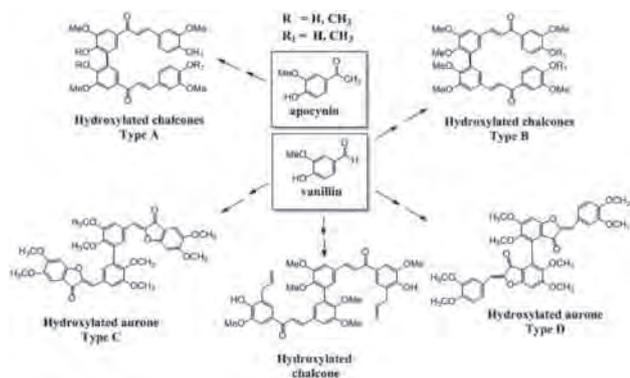
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1,3-Diaryl-2-propen-1-ones, commonly known as chalcones, precursors for the biosynthesis of flavonoids, have displayed remarkable variety of biological activities, among which antimalarial, anti-inflammatory, immunomodulatory and antitumoral.^[1] Aurones, (Z)-2-benzylidenebenzofuran-3-(2H)-ones, constitute a less-studied subclass of flavonoids. The spectrum of biological activity of this class of compounds has not been extensively studied despite it has been reported that they possess interesting inhibitory activities against a variety of enzymes and proteins.

In our laboratory, we are actively engaged in the synthesis and biological evaluation (e.g., antimelanoma cancer) of hydroxylated natural-like biphenyls.^[2] Hydroxylated biphenyl unit is embedded in many structures of bioactive natural products, and some of them are present in compounds of high biological relevance. Compared to 2-methoxy phenols, 3,3'-dimethoxy-2,2'-dihydroxybiphenyls generally manifest higher activity and less toxicity than the corresponding monomers. It is also known that the presence of a prenylated or unsaturated alkyl chain on flavonoids, including chalcones, can lead to a remarkable increase in bioactivity.^[3]

Herein, we report the synthesis, by base-catalysed Claisen–Schmidt condensation, of novel C₂-symmetry hydroxylated (Type A and B) chalcones based on a 2,2'-biphenyl scaffold and their corresponding monomers. Moreover, we report the preparation of new aurones (Type C and D) by aldol condensation of a substituted benzofuranone with a benzaldehyde derivative under mild conditions. Synthesis of chalcones and aurones, starting from natural compounds vanillin and apocynin, will be described.



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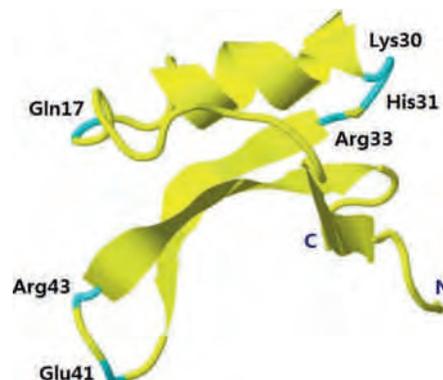
Identification of the Multi-point Binding Interactions between Human T1R2-T1R3 Receptor and Sweet Protein Brazzein by Site-Directed Mutagenesis

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The mechanism of interaction of sweet proteins with the sweet taste T1R2-T1R3 receptor has not yet been elucidated. To date, no tertiary structure has been reported for the sweet taste T1R2-T1R3 receptor. However, results from mutagenesis and chimeras of the human T1R2-T1R3 receptor suggest a multi-point interaction between sweet proteins and the sweet receptor. In order to identify a multi-point binding interaction between the sweet protein brazzein and the sweet taste receptor, 15 mutants of the residues in brazzein were constructed by site-directed mutagenesis. We found that mutations of Glu41 to Ala, Lys, or Arg in loop40–43 made the molecules significantly sweeter than brazzein. A similar pattern occurred at loop30–33, where mutation of the His31 to Arg significantly increased sweetness, while mutations at positions 30 or 33 in the immediate vicinity of this region significantly decreased sweetness. Mutations of Lys6 to Arg in β -strand I (residues 5–7) and Asp29 to Lys or Arg in the C terminus of one short α -helix (residues 21–29) significantly decreased sweetness. Conversely, the sweetness of the Glu36Asp mutant was approximately 3.5-fold higher than that of the wild-type brazzein, indicating that the negative charge and the length or orientation of the side chain of the amino acid at position 36 are important for eliciting sweetness. From these results, we suggest that the flexible loops containing His31 and Glu41 and Glu36 in β -strand III (residues 34–39) are the critical regions of the

molecule for eliciting sweetness, and charge and/or structure of the side chain of these residues play an important role in the multi-point binding interaction between brazzein and the sweet taste receptor.



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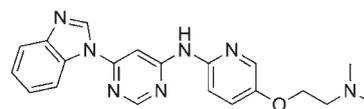
P515

Discovery of Potent Inhibitors of the HSF1 Stress Pathway: A Phenotypic Approach to Anticancer Drug Discovery

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Heat shock factor 1 (HSF1) is the master regulator of the heat shock response, a highly conserved protective mechanism. HSF1 is a transcription factor that drives the production of heat shock proteins (HSPs) such as HSP27, HSP70, and HSP90 as well as modulates the expression of hundreds of other genes critical for survival under a variety of stressors. Increased levels of HSPs in cancer cells allow them to manage the burden of drastic internal changes in core cellular physiology that are hallmarks of cancer, ultimately allowing them

to enhance proliferation and survival. A cell-based high-throughput screen of some 200,000 compounds was carried out by monitoring HSP72 protein expression in response to stress induced by a HSP90 inhibitor. A 4,6-disubstituted pyrimidine scaffold was identified and developed into potent inhibitors of the HSF1 pathway. This paper outlines the discovery, SAR development, and hit-to-lead medicinal chemistry campaign of this pyrimidine series.

P516

Design, Synthesis and Binding Affinity of Acetylcholine Carbamoyl Analogues

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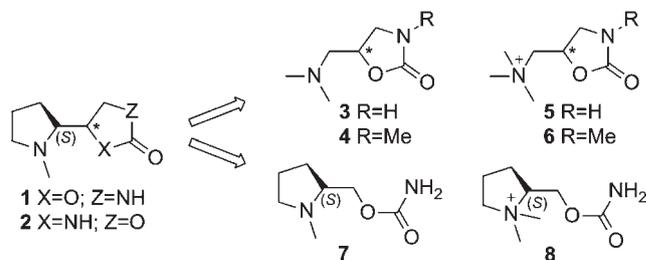
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Nicotinic receptors play an important role in the regulation of neurotransmission in the CNS and their dysfunction has been related to a number of severe brain pathologies. As a result, novel ligands for neuronal nicotinic receptors, in particular for the two major $\alpha 4\beta 2$ and $\alpha 7$ brain subtypes, may have a great potential for the treatment of several neurodegenerative disorders. Nicotine, the prototype of nicotinic agonists, has inspired the design of novel nicotinoids, mainly differing by the nature of the hydrogen-bond acceptor and π -electron-rich group (HBA/ π), which is 3-pyridinyl in nicotine, and/or by conformational flexibility. Recently, we have reported the synthesis and the binding affinity, for $\alpha 4\beta 2$ and $\alpha 7$ nicotinic subtypes, of all the stereoisomers of oxazolidinone bearing a 2-pyrrolidinyl substituent at the 5 position (**1**).^[1] The designed structure is characterized by the presence of two vicinal stereocentres that are connected by the sole bond, whose rotation is relevant to molecule conformation, and are placed in proximity of the critical cationic head with important consequences on the mutual disposition of N+ and HBA/ π . Unfortunately, all of the stereoisomers bind at $\alpha 4\beta 2$ nicotinic receptor with modest affinity. Therefore, in order to further investigate the interaction potential of oxazolidinone, we designed the positional isomer **2** and made the whole molecule less rigid by introducing an acyclic cationic head in place of pyrrolidinyl moiety (**3** and **5**). The effect of flexibility was also studied opening the oxazolidinone system but maintaining the pyrrolidinyl residue (**7** and **8**). Furthermore, we methylated the oxazolidinone nitrogen (**4** and **6**).

Herein, we report the synthesis, the nicotinic and muscarinic binding affinities and the docking analysis of the stereoisomers of compounds **1–8** with *S* configuration at the pyrrolidine stereocentre and *R* or *S* configuration at the oxazolidinone stereocentre. The structure–activity relationships are discussed also considering that

some of these compounds can be seen as rigidified analogues of carbachol, bethanechol, *N*-methylcarbamoylcholine and *N*-dimethylcarbamoylcholine.



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P517

Piperazine-2,3-dicarboxylic Acid Derivatives as Dual Antagonists of NMDA and GluK1-Containing Kainate Receptors

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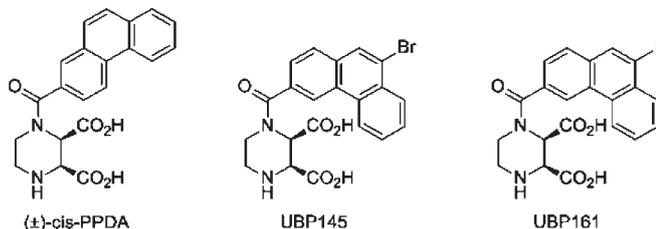
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Ionotropic glutamate receptors (*i*-GluRs) are L-glutamate-gated ion channels that mediate fast synaptic transmission in the CNS. The three families of *i*-GluRs are named after compounds by which they are selectively activated: kainate [(2*S*,3*S*,4*S*)-3-carboxymethyl-4-isopropenyl-pyrrolidine-2-carboxylic acid], AMPA [(*S*)-2-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid], and NMDA [*N*-methyl-D-aspartic acid] receptors. *i*-GluRs are tetrameric in structure with kainate receptors (KARs) being assemblies of GluK1–5 subunits. AMPA receptors (AMPARs) are assemblies of GluA1–4 subunits, while NMDA receptors (NMDARs) can be assembled from GluN1, GluN2A–D and in some areas of the CNS, GluN3A and GluN3B subunits. Both NMDARs and KARs have been implicated in a variety of neurological conditions including epilepsy and schizophrenia and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. In addition, GluN2D-containing NMDARs and GluK1-containing KARs are important elements of pain signalling in the dorsal horn and make attractive targets for drug development.

(2*S**,3*R**)-1-(Phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid (PPDA) is a potent NMDAR antagonist with weak selectivity toward the GluN2D subunit.^[1,2] Whilst investigating its selectivity profile across glutamate receptor subtypes, PPDA was found to

display antagonist activity against GluK1-containing KARs. To understand the structural elements required for selective activity at GluK1-containing KARs and GluN2D-containing NMDARs, various structural analogues of PPDA were synthesised and characterised across a range of NMDAR and KAR subtypes.^[3] We will present data from this SAR study and results from modelling studies using Glide to dock PPDA analogues into the ligand binding domains of GluN2D and GluK1. Of the compounds synthesised, UBP145 was found to be 7–10-fold selective for GluN2D versus GluN2A and GluN2B and >10-fold selective for GluK1 over GluK2. In addition, one of the most potent KAR antagonists in this series (UBP161) proved to be selective for GluK1 over GluK2 and GluK3 and may, therefore, have utility as a pharmacological tool for probing the function of GluK1-containing KARs.^[3]



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P518

Discovery of Novel [16,17]Isoxazoline Derivatives of Prednisolone and 6- α -Fluoro-isoflupredone as Glucocorticoid Receptor (GR) Ligands

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Glucocorticoids are highly effective in the control of chronic inflammatory diseases such as asthma, where they have become the mainstay of therapy. Glucocorticoid receptors are specific cytoplasmic transcription factors that mediate the biological actions of glucocorticoid (Ito, Chung et al., 2006). A series of structurally novel isoxazoline glucocorticoid receptor (GR) modulators, whose general structure is shown below, has been identified starting from prednisone acetate and difluprednate.

Isoxazoline derivatives were synthesized by using the 1,3-dipolar cycloaddition of the corresponding nitrile oxide. In the present study, all of these compounds were extensively profiled in vitro (binding, functional and selectivity assays), and results compared with the best in class compounds, fluticasone furoate and budesonide. The

relatively weak GR binding affinity of compounds with X,Y=hydrogen was improved (10-fold) with the incorporation of fluorine in 6,9 position. Furthermore, the derivatives with R¹=bromine showed sub-nanomolar affinity for GR, nanomolar potency and 95% efficacy in the functional assay (nuclear translocation). The structure–activity relationship of these isoxazoline derivatives will also be discussed together with their potential binding mode in the GR binding site, identified by performing docking experiments.



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P519

Identification of Highly Potent and Selective Polo-Like Kinase 1 Inhibitors

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Polo-like kinase 1 (PLK-1) is a serine/threonine protein kinase involved in different stages of mitosis with roles in centrosome maturation, bipolar spindle formation, chromosome separation and cytokinesis. The expression, activity and localization of PLK-1 is dynamically regulated during the cell cycle and PLK-1 protein levels increase from the late S-phase to mitosis. PLK-1 is overexpressed in a variety of human tumours including lung, colon, stomach, breast, ovary, head and neck, and melanomas where often correlates with poor prognosis. Inhibition of PLK-1 expression by siRNA or antisense oligonucleotides further validates PLK-1 as an attractive target for anticancer drug therapy. Here, we report the identification and the synthesis of highly potent PLK-1 inhibitors belonging to the dihydropteridinone class. The compounds were found to inhibit the PLK-1 enzyme in the low nanomolar range and to have good selectivity versus a large panel of kinases comprising the PLK-2 and PLK-3 isoforms. Co-crystal structure of methylated construct of

PLK-1 (36–345) with a representative inhibitor was also determined. The compounds showed mechanism of action in agreement with PLK1 inhibition and antiproliferative activity on tumour cells in the nanomolar range. The most interesting compounds were profiled for ADME properties in vitro and in vivo in mouse where oral bioavailability was shown.

P520

Discovery of Novel Dual Angiotensin II and PPAR Gamma Modulators

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Metabolic syndrome is a cluster of metabolic disorders such as high blood pressure, obesity, insulin resistance, and dyslipidaemia and constitutes a major risk factor for cardiovascular diseases. Telmisartan (Micardis®), a highly selective angiotensin II AT₁ receptor blocker (ARB) used for treatment of hypertension, was found to exhibit unique effects on levels of serum glucose, serum triglycerides and on insulin sensitivity. In vitro and preclinical in vivo experiments have shown PPAR gamma activation to be directly linked to these benefits. Compounds possessing the dual pharmacology of an AT₁ receptor antagonist and PPAR gamma modulator could potentially treat several cardiovascular risk factors in patients with metabolic syndrome.

Starting from the chemical structure of telmisartan, a rational drug design program was initiated to discover new potent dual angiotensin II receptor antagonists and selective PPAR gamma modulators. Here, we describe the synthesis and the structure–activity relationship of this new series. In addition, the in vivo pharmacology and pharmacokinetic parameters of the compound selected will be provided.

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P521

Lipophilicity of Some Active *Mycobacterium tuberculosis* Isoniazid Derivatives: A Comparative Study between Octanol–Water ($\log P_{\text{oct/w}}$) and Micelle–Water ($\log K_p$) Partition Coefficients

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Tuberculosis (TB) is a worldwide infectious disease with extremely high mortality levels.^[1] Despite the urgent need for new antitubercular drugs, driven by the increasing number of multidrug-resistant strains of *Mycobacterium tuberculosis* (*M. tb*), no antibacterial therapeutics regimes have emerged over the last decade. The large costs involved in the development of new drugs, have led to the development of quantitative structure–activity relationships (QSARs) aiming at the prediction of biological activity. Some of us have previously reported QSAR models for biological activity against *M. tb* of hydrazide derivatives, using multilinear regressions, an approach which has guided the synthesis of some promising new isoniazid derivatives.^[2]

Being generally accepted that pharmacological processes involve penetration, binding, and activation steps, the interaction of new drug-like candidates with membranes, the first barrier, may determine their activity. Thus the lipophilicity of compounds with predicted high activities is extremely useful in a preliminary screening of potential new drugs.

Lipophilicity, conventionally expressed in the pharmaceutical industry in terms of the octanol–water partition coefficient ($P_{\text{oct/w}}$), has also been evaluated in terms of partition coefficients (K_p) toward membrane mimetic systems (micelles/liposomes) in an attempt to account for specific interactions and for the anisotropic characteristics of the lipid membrane bilayer. Several authors^[3–5] have correlated $\log K_{p(\text{NaDS/w})}$ (sodium dodecyl sulfate micelles/water) to $\log P_{\text{oct/w}}$ data for several monofunctional solutes. In this study some isoniazid derivatives active against *M. tb*. also show a linear dependence between these parameters, being positive and negative deviations from this correlation discussed and reasoned in terms of molecular structure and solute localization in the micellar pseudo phase.

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P522

Discovery of Orally Active Selective Inhibitors of Plk1

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Members of the Plk family of Ser/Thr protein kinases play important roles in the entry to, progression through, and exit from cell mitosis. Plk1 is overexpressed in a variety of cancers and these high expression levels often correlate with poor prognosis. Plk1 is therefore considered to be a good target for the development of novel antimetabolic cancer therapies. As part of our efforts to discover new cancer therapeutics, we developed a high-throughput screen (HTS) against Plk1. This led to the identification of a series of pan-Plk inhibitors based on the pyrrolopyridine scaffold. A structure-based drug-design approach enabled us to optimize the HTS hits to obtain a series of inhibitors that were selective for Plk1 against other members of the Plk family. These compounds demonstrated potent inhibition of cellular proliferation in vitro and tumour growth in animals following oral administration.

P523

Novel and Highly Selective Inhibitors of Fibroblast Activation Protein (FAP)

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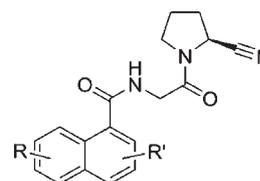
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Fibroblast activation protein (FAP) is a serine protease selectively expressed on activated fibroblasts on over 90% of all human carcinomas.^[1] Several publications claim an important role for FAP in tumor growth and proliferation.^[1,2] High expression of FAP also suggests importance in other pathological processes that involve remodeling of the extracellular matrix such as fibrotic disease, wound healing, keloid formation and osteoarthritis.^[3,4]

Starting from (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-1-naphthamide, a series of substituted quinolinecarboxamide-*N*-glycyl-(2*S*-cyano)pyrrolidines were synthesized as novel inhibitors of FAP (scaffold shown). The influence of the substitution pattern on the quinoline ring and the nitrogen position in the quinoline ring was investigated.

Currently there are no FAP inhibitors with reported selectivity towards prolyl oligopeptidase (PREP) and dipeptidyl peptidases (DPPs). Our inhibitors displayed inhibitory potency in the low nanomolar range and showed good to excellent selectivity with respect to the proline-selective dipeptidyl peptidases (DPPs) DPP IV, DPP9, DPP II and PREP. The plasma stability, kinetic solubility and log*D* of selected compounds were found to be satisfactory.



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P524

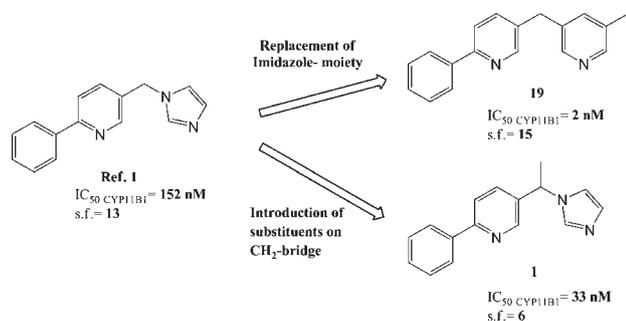
Optimizations of 2-Phenyl-5-(1-imidazolymethyl)pyridine Lead to Significant Improvement of Potency and Selectivity as CYP11B1 Inhibitors

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Cushing's syndrome and Metabolic syndrome are diseases which are associated with elevated cortisol levels.^[1] Concerning medical therapy a promising target to decrease cortisol formation is steroid-11 β -hydroxylase (CYP11B1), an adrenal CYP enzyme, which directly affects cortisol production by catalyzing the hydroxylation of deoxycortisol in 11 β -position.^[2] Inhibitors of cortisol biosynthesis that are already in clinical use, like ketoconazole, etomidate and metyrapone, are unselective. This means they inhibit a broad range of CYP enzymes and therefore show a lot of side effects.^[1] Due to the fact that the homology between CYP11B1 and CYP11B2 (aldosterone synthase) is very high (93%) the development of a selective and potent CYP11B1 inhibitor is challenging.^[3] Starting from the unselective CYP11B1 inhibitor, R-Etomidate, a novel selective compound (**Ref. 1**) had been identified.^[4,5]

Here, we report on further optimization of **Ref. 1** (scheme shown). The bioisostere exchange of the CH₂-bridge with O/NH and the change of the pyridine core with other heterocycles led to a loss of activity. However by introducing different substituents on the CH₂-bridge, **1** was obtained with increased activity (IC₅₀CYP11B1=33 nM) but slightly decreased selectivity factor (s.f.: IC₅₀CYP11B2/IC₅₀CYP11B1=6). Finally, the exchange of the imidazole moiety to 5-methylpyridine-3-yl led to a very potent and more selective compound **19** (IC₅₀CYP11B1=2 nM; s.f.=15). Furthermore, the substance showed no inhibition of the androgen-forming CYP17 and aromatase (estrogen synthase, CYP19). This compound is considered as a promising candidate for further in vivo evaluations.



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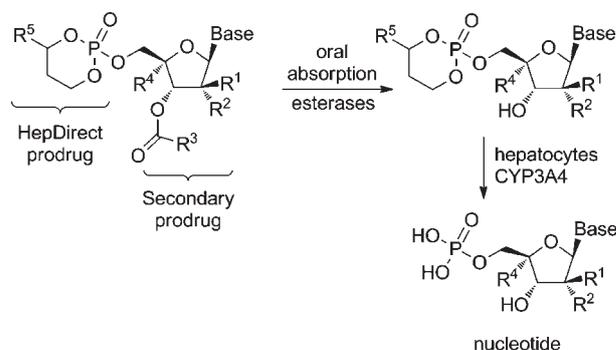
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P525

Discovery of Liver-Targeting Nucleotide NS5B Polymerase Inhibitors using HepDirect Prodrug Technology

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HepDirect™ technology represents a novel class of cytochrome P450-activated prodrugs capable of targeting certain drugs to the liver for the potential benefit on drug efficacy and safety, and the technology has additional advantages for targeting nucleotide-based antiviral drugs since in vivo conversion of a nucleoside to the active nucleotide can be rate limiting.^[1] A series of novel HepDirect prodrug compounds were synthesized and optimized as nucleotide NS5B polymerase inhibitors designed to have improved clinical efficacy and safety. The cyclic phosphate prodrug moiety was designed as a substrate of CYP3A4, an enzyme mainly expressed in the liver, for an oxidative cleavage to provide the nucleotide for liver targeting, and the secondary esterase prodrug moiety improved oral bioavailability (see figure). Early lead compound MB11362 demonstrated clinical efficacy in chronic hepatitis C patients in a proof-of-concept study.^[2] Synthesis, animal pharmacokinetics, liver-targeting profile, and SAR of the series will be discussed.

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P526

Identification and Optimization of Novel Triazolo-indazole IRAK-4 Inhibitors using Structure-Based Design, Physicochemical and ADME Properties Modulation

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Interleukin-1-receptor-associated kinases (IRAKs) are involved in the signal transduction pathways mediated by interleukin-1-receptors (IL-1R) and Toll-like receptors (TLRs). IRAK family of Ser/Thr kinases is composed of four isoforms: IRAK-1, IRAK-2, IRAK-4 and IRAK-M. Among them, only IRAK-1 and IRAK-4 possess kinase activity, and IRAK-4 is thought to be the initial protein kinase activated downstream of the interleukin-1 (IL-1) receptor and all toll-like-receptors (TLRs) except TLR3. Activation of IRAK-4 initiates the signaling in the innate immune system via the rapid activation of IRAK1 and slower activation of IRAK2. IRAK4-deficient mice are protected from inflammation in two models of joint inflammation, are resistant to LPS- and CpG-induced septic shock and do not produce IL-6 or TNF- α upon IL-1 or LPS challenge. IRAK-4 deficient patients exhibit functional defects in TLR/IL-1R signaling pathway. Those data among others, suggest that blockade of IRAK-4 by small-molecule inhibitors could be an approach to develop new drugs to treat autoimmune and inflammatory diseases such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease (IBD) or systemic lupus erythematosus (SLE). A new indazole hit series was identified via a high-throughput screening on IRAK-4. X-ray structure in IRAK-4 of representative analogues allowed a structure-based optimization of potency and selectivity profile of this chemical series. Physicochemical and ADME parameters optimization allowed the identification of a new indazole derivative demonstrating efficacy in an LPS-induced cytokine release model in mice when administered po at 30 mpk.

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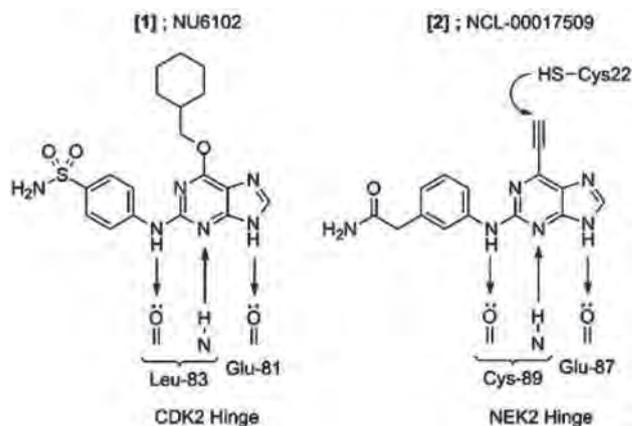
2-Arylamino-6-ethynylpurines as Potent Irreversible Inhibitors of the Mitotic Kinase Nek2

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The serine/threonine kinase Nek2 is of considerable interest as a potential drug target for cancer, owing to its important role in the cellular mitotic machinery and abnormal expression in a number of human malignancies.^[1] As a consequence, efforts directed towards the design and synthesis of Nek2 inhibitors have resulted in the identification of both ATP-competitive and irreversible inhibitors of this kinase.^[2,3] Screening of a series of purine derivatives, originally developed as CDK2 inhibitors, identified a number of compounds with modest Nek2 inhibitory activity (e.g., NU6102 (**1**); CDK2, IC₅₀=5.0 nM; Nek2, IC₅₀=12 μ M).^[4] Further studies, guided by crystal structures of Nek2 in complex with a range of purine-based inhibitors enabled the design of potent irreversible inhibitors as exemplified by the 6-ethynylpurine derivative NCL-00017509 (**2**). Importantly, the purine heterocycle of **2** maintains the key triplet of hydrogen bond interactions with the kinase hinge region, and positions the 6-ethynyl substituent proximal to Cys22, thereby facilitating a covalent Michael reaction with the thiol.



Enzyme kinetic studies demonstrated a time-dependent inhibition of Nek2 by **2** and related compounds, consistent with the irreversible nature of their interaction within the ATP-binding domain. A crystal structure of **2** in complex with Nek2 confirmed covalent modification of Cys22 thiol leading to an adduct in which a CH=CH group links the purine to Cys22-S. The activity of **2** as a potent kinase-selective

irreversible Nek2 inhibitor ($IC_{50}=56$ nM), combined with promising drug-like properties, prompted further investigations with this chemotype. The synthesis, structure–activity relationships and structural biology of selected 2-arylamino-6-ethynylpurine Nek2 inhibitors will be discussed.

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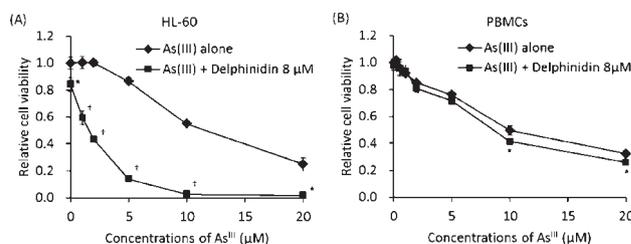
Enhanced Cytotoxic Effects of Arsenite in Combination with Delphinidin against a Human Leukemia Cell Line, HL-60

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Administration of arsenic trioxide (arsenite, As^{III}), an arsenic derivative, has demonstrated a remarkable efficacy in the treatment of relapsed and refractory acute promyelocytic leukemia (APL) patients. In order to understand the mode of action of As^{III} and provide an effective treatment protocol for individual APL patients, our research groups have conducted the studies on the pharmacokinetics of As^{III} in APL patients using biological samples such as blood, cerebrospinal fluid, and bone marrow.^[1–3] We also demonstrated that aquaporin 9 and multidrug resistance-associated protein 2 contributed to differential sensitivity to arsenite among primary human-derived normal cells,^[4] based on a study using a unique in vitro cell-culture system comprising primary culture chorion and amnion cells established in our laboratory.^[5–6] These findings provide a new insight into clinical applications of As^{III} , and may contribute to better therapeutic protocols.^[7] Due to a remarkable clinical efficacy of As^{III} -based regimens against acute promyelocytic leukemia (APL), the effect of As^{III} has been investigated in other cancer cells, suggesting that cytotoxic effects of As^{III} are not restricted to APL cells but also in certain types of cancer cells, such as prostate and ovarian carcinomas. However, low sensitivity of these cells to As^{III} is still a serious concern and hamper its future clinical applications. In fact, the human acute myeloid leukemia cell line HL-60 is also reported to show resistance to As^{III} . In this regard, it is interesting to note that anthocyanidins including delphinidin show obvious cytotoxic effects in various solid

tumor cells. Therefore, we hypothesize that the combination of As^{III} and delphinidin is able to sensitize As^{III} -resistant cells, HL-60, to As^{III} . In order to prove the hypothesis, we investigated the effects of As^{III} and delphinidin, alone or in combination, on HL-60 cells and healthy human-derived peripheral blood mononuclear cells (PBMCs). Results demonstrated for the first time that delphinidin selectively inhibited the growth of HL-60, but minimal cytotoxic effect on PBMCs, and sensitized the cells to As^{III} , resulting in the enhanced As^{III} cytotoxicity (see figure). Moreover, apoptotic as well as necrotic events appear to be involved in the cytotoxic effects. Delphinidin-induced sensitization of HL-60 to As^{III} was caused by the reduction of intracellular glutathione content ([i]GSH), which might be mediated through its inhibitory effect on NF- κ B activity. These observations suggest that delphinidin is able to improve clinical efficacy of As^{III} by overcoming the defect of As^{III} in the unexpected increment in [i]GSH. Furthermore, sensitization of HL-60 cells to As^{III} achieved by the combination with delphinidin could reduce As^{III} dosages that contribute to minimize side effects.



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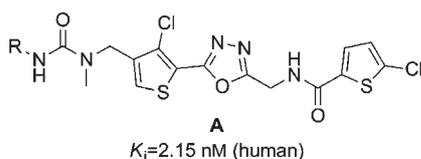
Novel Factor Xa Inhibitor, 1,3,4-Oxadiazole Derivatives: Design and Synthesis Based on Co-crystal Structure Analysis

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Factor Xa (FXa) is known as one of the most important coagulation factors since it is located at the convergence of the intrinsic and extrinsic coagulation pathways. Heparin and its derivatives are commonly used as anticoagulants and antiplatelet agents; however, there remain demands for orally administrable FXa inhibitors. Although the first generation FXa inhibitors like DX-9065a containing

one or two amidine moieties showed promising inhibitory activities, their bioavailabilities were not acceptable as oral drugs. Since then, many pharmaceutical organizations have been focusing on the development of FXa inhibitors without amidine structures. Rivaroxaban was launched as the first-in-class orally available FXa inhibitor. Several orally available inhibitors are under clinical development. Here we report a novel potential FXa inhibitor (**A**), which has no amidine moiety and highly potent inhibitory activity ($K_i=2.2$ nM). The structure avoided a chiral center and is simple and unique for further development. Compound **A** was designed from already reported several inhibitors, and tuned out to overlap with their key structures.



In the process of the development of compound **A**, we studied the structure–activity relationship of compound **A** derivatives. As a result, we found that the 1,3,4-oxadiazole-*ortho*-substituted aromatic ring–urea substructure was essential to show the promising inhibitory potency. We also found that the derivatives could use two different unexpected binding sites, which depended on their substituents on the aromatic ring according to the co-crystal structure analyses with human FXa. The results assisted us to design and synthesize some more developed compounds that could use both binding side in order to earn both benefits with introduction of two substituents on the aromatic ring at the same time. Finally, we achieved to obtain highly potent FXa inhibitors whose K_i value is less than 1 nM.

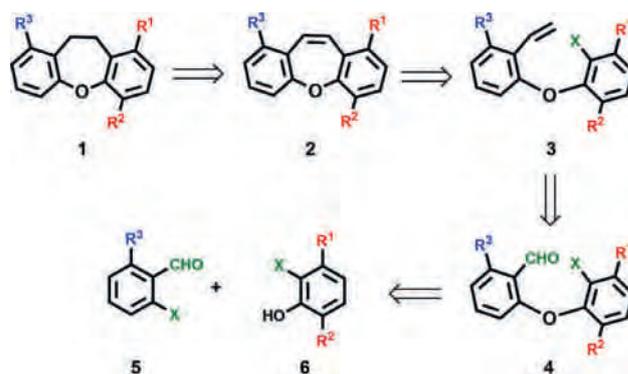
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Studies on the Synthesis of Dibenz[*b,f*]oxepins

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The dibenz[*b,f*]oxepin system is the basic skeleton of a number of biological active natural products and medicinal relevant compounds.^[1] Recently, novel dihydrodibenz[*b,f*]oxepins isolated from *Bauhinia purpurea* exhibiting antimalarial, antimicrobial, antifungal and cytotoxic activities were reported.^[2] The interesting biological activities of these compounds has stimulated their synthesis and some approaches have been described,^[3] but further studies are needed for structural-biological activity correlations. Following our studies on the preparation of bioactive heterocyclic quinones,^[4] herein, we describe results on the synthesis of dihydrodibenz[*b,f*]oxepin (**1**). Our initial retrosynthetic analysis included study of the intramolecular Heck reaction and Ullmann-type biaryl ether preparation.



The reaction of benzaldehydes **5** with phenols **6** were carried out in DMSO/ Cs_2CO_3 under microwave irradiation for 5 min at 90 °C to afford diaryl ethers **4** in 80–90% yield. Wittig reaction of **4** with methyltriphenylphosphonium bromide and potassium *tert*-butoxide in THF produced *ortho*-vinyl diaryl ethers **3** in high yields. The intramolecular Heck reaction of **3** using $\text{Pd}(\text{OAc})_2$, KOAc and TBAB in DMF is under study. In another approach, based on an intramolecular Ullmann-type biaryl ether formation dihydrodibenz[*b,f*]oxepin **1** ($\text{R}^1=\text{R}^2=\text{R}^3=\text{H}$) was obtained in six steps with 31% total yield.

Acknowledgements: We are grateful to FONDECYT (grant 1110749) and VRI (grant 2010/4).

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P531

Sootependial, a New 3,4-*seco*-Cycloartane from a Thai *Gardenia* Plant, Induces Apoptosis in Liver Cancer Cells and Angiogenic Inhibition

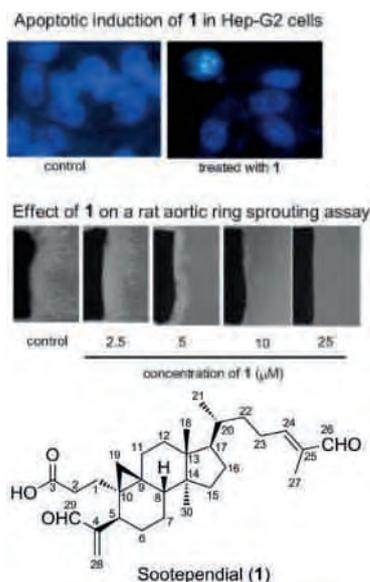
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Ring-A 3,4-*seco*-cycloartane triterpenes have been mainly found in plants belonging to the genus *Gardenia* (Rubiceae). Many of them have been found to exhibit cytotoxic activity towards various cancer cell lines. We aimed to determine 3,4-*seco*-cycloartane from Thai *Gardenia* spp. potential for cancer leads and to explore the detailed mechanism. In this study, a new 3,4-*seco*-cycloartane triterpenes, sootependial (**1**) was isolated from bud exudate of Thai *G. sootepensis*, and its struc-

ture was elucidated on the basis of spectroscopic data. Sootependial (**1**) showed potent cytotoxicity selective to human liver cancer cells (Hep-G2) in MTT assay and antiangiogenic activity in ex vivo model (a rat aortic ring sprouting). Treatment with **1** exerted growth inhibition through G1 arrest and actively induced apoptosis of Hep-G2 cells. Its induction of apoptosis was accompanied by a reduction of Bcl-2 level. Moreover, its angiogenic effect was found to occur mainly by suppressing endothelial cell proliferation and tubule formation, suggesting the potential of **1** as a lead compound for cancer treatment.



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Synthesis and Kinetic Studies on the Inhibition of GABA-AT of Some Structural Analogues of GABA, Pregabalin and Baclofen

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 José Trujillo-Ferrara, Mario Fernandez-Zertuche

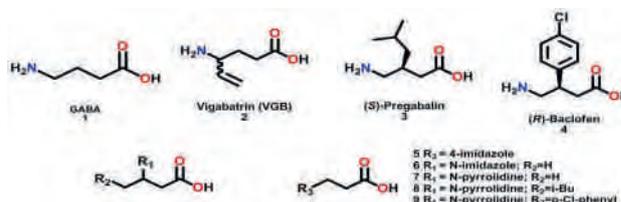
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γ -Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central nervous system.^[1] GABA levels are regulated by the enzyme L-glutamate descarboxylase (GAD), an enzyme involved in the biosynthesis of this neurotransmitter and the catabolic enzyme, γ -aminobutyric acid aminotransferase

(GABA-AT).^[2] Inhibition of GABA-AT increase the concentration of GABA in the brain, a fact that may have several therapeutics applications in common neurological disorders.^[3] Here, we show the design, synthesis and determination of inhibitory potential on GABA-AT of some structural analogues of GABA, pregabalin and baclofen. We accomplished the synthesis of compounds **5–9**, as well as the determination of some kinetic parameters on the enzyme GABA-AT.

We evaluated the inhibition (%) of the analogues **5–9** at 0.8 μ M and compared our results with respect to GABA (control) at the same concentration. Analogues with inhibition percentage ranges over 40% were analyzed in greater depth to determine the kinetic parameters K_m , r_{max} , K_i and αK_i , to establish the kind of inhibition. This study was conducted at λ_{max} of 340 nm at 25°C for 30 min and different concentrations of inhibitor (2.0, 1.0, 0.5 and 0.25 μ M). Analogues **8** and **9** showed the highest inhibition percentage, 48 and 70% respectively, suggesting that the presence of a substituent at the β position to the carbonyl plays an important role in the inhibitory potential. The data were analyzed by the Lineweaver–Burk method for determining the values of K_m and r_{max} for both **8** and **9**, graphically. In both cases it was determined that it is mixed inhibition. According to the obtained values of K_i and αK_i , analogue **8** is more akin to the ES complex ($\alpha K_i=0.5675 \mu$ M), while compound **9** is more akin to the enzyme ($K_i=0.815 \mu$ M). Analogues **8** and **9** might be good candidates for neuroprotective seizures.

Compd	Inhibition [%] (prelim. test)	r_{max} [μ mol/min mg]	K_m [μ M]	K_i [μ M]	αK_i [μ M]
GABA	0	0.5148	0.4423	–	–
VGB	41	0.5189	0.4423	0.497	–
5	19	ND	ND	ND	ND
6	18	ND	ND	ND	ND
7	41	ND	ND	ND	ND
8	48	0.5363	0.4838	1.759	0.5675
9	70	0.4344	0.3192	0.815	1.5341



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Synthetic Studies and Biological Evaluation of Oxazole Derivatives as a Novel Scaffold of HIV-1 Inhibitors

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Since the discovery of the human immunodeficiency virus (HIV) as the causative agent of the acquired immunodeficiency syndrome (AIDS) in 1983, the virus has rapidly spread around the world and HIV/AIDS is currently considered a pandemic.^[1] According to UNAIDS 2009 report [Data.unaids.org], worldwide some 60 million people have been infected, with some 25 million deaths, and 14 million orphaned children in southern Africa alone since the epidemic began. This major global health threat triggered intensive drug discovery efforts and the first FDA-approved antiretroviral drug, AZT, was available in 1987. To date, 25 anti-HIV drugs belonging to 6 different inhibitor classes have been approved by FDA for the treatment of HIV infection, including nucleoside or non-nucleosides reverse transcriptase inhibitors (NRTIs/NNRTIs), protease inhibitors (PIs), integrase inhibitors, entry and fusion inhibitors.^[2] The introduction of highly active antiretroviral therapy (HAART)—a regimen combining 3–4 antiretrovirals from different inhibitor classes—has significantly improved the life quality of patients by delaying the progression of the disease and reducing disabilities, transforming HIV/AIDS into a chronic manageable disease. However, the lack of an effective and safe vaccine, the emergence and spread of drug-resistant viral variants^[3] and the inability of current regimens to eradicate the virus enforce the continuous development of novel anti-HIV drugs.

As a part of our HIV program aimed at the discovery of novel antiretrovirals via HTS using a cell-based assay, we successfully identified a novel class of 5-(phenethylamino)-2-phenyloxazole-4-carbonitrile (AOZ) anti-HIV-1 agents. Initial structure–activity relationship (SAR) studies which had been done in the focused library containing around 80 compounds revealed that the original scaffold itself was a very important moiety for the retention of anti-HIV activity. In the mode-of-action study of this small molecule, it was determined that the relevance of the target to the small molecule's anti-HIV activity was profoundly linked to the inhibition of HIV reverse-transcriptase. This work is expected to provide valuable information for the discovery of a novel anti-HIV-1 scaffold from target-free cell-based assay system.

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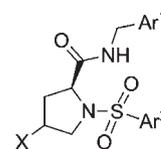
Identification of a BBB-Penetrating TRPA1 Antagonist Showing Efficacy In Vivo

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Transient receptor potential ankyrin 1 (TRPA1) is a calcium-permeable nonselective cation channel which is selectively expressed in nociceptors. TRPA1 has evolved to respond to a wide range of exogenous chemical irritants. Sustained endogenous activation of TRPA1 has been shown to be involved in several chronic pain animal models, such as streptozotocin-induced diabetic neuropathy, CFA-induced inflammation and postoperative pain. According to recent reports, TRPA1 antagonists alleviate pain in different pain models in rat and mice, suggesting that such compounds may have applications in the treatment of pain.^[1–3]

A medium-size screening campaign identified some derivatives of L-proline (see figure) as antagonists of human and rat TRPA1, showing no tendency of agonism. Closer examination of the hits revealed some undesired properties, such as low metabolic stability and mechanism-based CYP inhibition, reducing the drug-likeness of the hits, and restricting their usefulness as pharmacological tool compounds. The modifications which led to more stable antagonists, and the consequences in vivo, are presented and discussed.



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P535

Targeting GSK3 from *Ustilago maydis*: Type-II Kinase Inhibitors as Potential Antifungals

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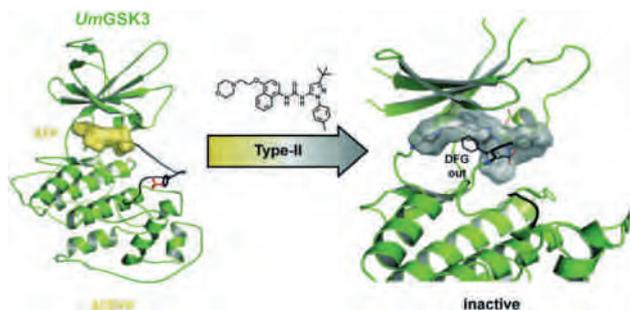
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Protein kinases are key enzymes in the complex regulation of cellular processes in almost all living organisms. For this reason, protein kinases represent attractive targets to stop the growth of eukaryotic pathogens such as protozoa and fungi. However, using kinase inhibitors to fight against these organisms bears several challenges since most of them are unselective and will also affect crucial host kinases. Here we present the X-ray structure of glycogen synthase kinase 3 from the fungal plant pathogen *Ustilago maydis* (*UmGSK3*) and its inhibition by type-II kinase inhibitors. Despite the high sequence homology between the human and the fungal variant of this vital kinase, we found substantial differences in the conformational plasticity of their active sites. Compounds that induced such conformational changes could be used to selectively inhibit the fungal kinase version. This study serves as an example on how species-specific selectivity of inhibitors can be achieved by identifying and addressing the inactive state of a protein kinase. In addition to this, our study gives interesting insights into the molecular plasticity of *UmGSK3* by revealing a previously unknown inactive conformation of this important kinase family.



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Synthetic Studies on Cyclopropane-Containing GABA Analogues

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Here, we report our synthetic efforts toward the development of novel GABA analogues such as dysibetaine CPa, CAMP, and TAMP, that are conformationally constrained by a cyclopropane framework. The cyclopropane core was constructed by addition of sulfonium ylide or diazomethane to unsaturated esters. The asymmetry was introduced either by chiral auxiliary method employing optically active menthol, or asymmetric solvolysis using organocatalysis. The structural diversity was gained by diastereomeric evolution as well as various substituents on the amine functionality. Six analogues have been so far successfully synthesized, and the synthesis of other four analogues is currently underway in our laboratory. The molecular design as well as the details on the synthetic study will be discussed.

P537

Cytotoxic and Anti-angiogenic Effect of Merulin C, a Sesquiterpene Endoperoxide from a Thai Mangrove-Derived Fungus

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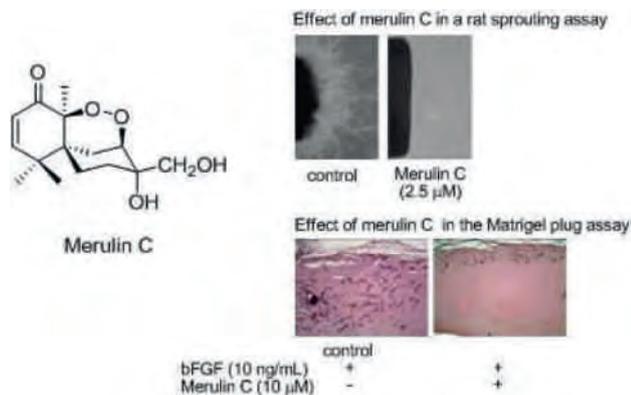
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The work described is part of our ongoing efforts to investigate natural products with potential for use as cancer treatments. Angiogenesis is the formation of new blood vessels from pre-existing blood vessels, and it is involved in various pathological states including tumor growth, diabetic retinopathy, and age-related macular degeneration. Inhibition of angiogenesis is an important target for cancer treatment because not only solid tumor growth but also metastasis is facilitated through angiogenesis. Recently, we have reported that a new sesquiterpene endoperoxide, merulin C, from a Thai mangrove-derived fungus showed cytotoxicity against human breast (BT474) cancer cells with an IC_{50} value of 1.57 $\mu\text{g}/\text{mL}$. In the present study, their anti-angiogenic activity was evaluated. Ex vivo angiogenesis model, a rat aortic sprouting assay, was first performed to determine their activity. Results revealed that merulin C displayed potent activity with complete inhibition at a dose of 2.5 μM and inhibited in vitro angiogenesis by mainly suppressing human

umbilical vein endothelial cell (HUVEC) proliferation and migration. In addition, our finding could indicate that mode of action of merulin C occurred via inhibition of Erk1/2 phosphorylation. Therefore, merulin C might be a novel inhibitor of angiogenesis, suggesting that it may be important for providing a model molecule for the discovery of potential new agent useful for angiogenesis-dependent diseases, especially tumor treatment and prevention.



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P538

Design, Synthesis and Biological Evaluation of 4-Cycloalkyl-Substituted 3,4-Dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxides Acting as AMPA Potentiators

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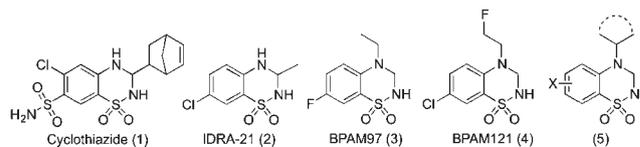
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Alzheimer's disease (AD) represents one of the greatest health problems in industrialized countries, in view of the aging population. Only four drugs are currently approved against AD. As these drugs have a limited time of efficacy, the need is great for additional innovative AD treatments. Amongst the numerous strategies currently investigated to avoid AD progress stands the promising approach based on the potentiation of AMPA receptors, which could possibly either attenuate or reverse the cognitive decline. This pharmacological class has also been proposed for the management of schizophrenia or depression.

Contrary to AMPA agonists that may cause severe adverse effects such as neurotoxicity, the AMPA potentiators (AMPA-PAMs) seem to be a more interesting and less toxic category of compounds because of their properties: they are allosteric positive modulators able to potentiate the AMPA signals in the presence of glutamate, having no effect on the receptor in the absence of the endogenous neurotransmitter. The AMPA receptors have represented an interesting target to develop cognitive enhancers since this subtype of ionotropic glutamate receptors (iGluRs) were shown to be involved in the expression and the maintenance of long-term potentiation. The interest of this pharmacological class has recently been reinforced considering its neuroprotective and neuroplastic enhancing effects linked to the stimulation of BDNF release.

For the last two decades, 1,2,4-benzothiadiazine 1,1-dioxides have been investigated to discover new AMPA-PAMs. Starting from the structure of cyclothiazide (1) and IDRA-21 (2), our team previously synthesized in vitro active 3,4-dihydro-2H-benzothiadiazine 1,1-dioxides from which emerged BPAM97 (3).^[1] Considering the poor pharmacokinetic behaviour of this first lead compound, the introduction of fluorine atoms was tempted as a lead optimization strategy and resulted in the development of BPAM121 (4).^[2] In the attempt to further enhance the in vitro activity as well as the in vivo efficacy of the previously described compounds, the present work focused on the insertion of new alkyl and cycloalkyl chains at the 4-position of the thiadiazine ring (5). Biological evaluations were realized in vitro on *Xenopus* oocytes expressing AMPA receptors. The in vitro screening permitted to select several compounds for in vivo studies.



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P539

Anti-infectives with Novel Mode of Action: Development of PqsD Inhibitors to Interrupt *P. aeruginosa* Cell-to-Cell Communication

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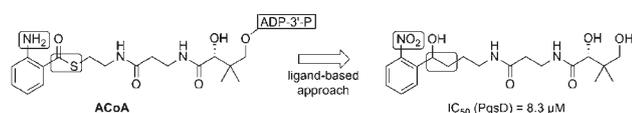
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P. aeruginosa coordinates group behaviours via a cell density-dependent cell-to-cell communication system known as quorum sensing (QS).^[1] It employs a characteristic *pqs* QS system that functions via

the signal molecules PQS and its precursor HHQ. By interaction with their receptor PqsR virulence genes and biofilm formation are controlled. PqsD is an essential enzyme in the biosynthesis of HHQ,^[2] which makes PqsD an attractive target for drug development, since reduction of HHQ levels leads to limited systemic bacterial dissemination and reduced mortality in infected mice.^[3]

In a ligand-based approach, analogues of the natural substrate anthraniloyl-CoA (ACoA) and mimics of the corresponding transition state have been prepared in a 12-step synthesis. Thereby an inhibitor with moderate activity has been identified.

To improve drug-likeness, we simplified the molecule by shortening and rigidization of the flexible side chain. Interestingly, these fragments showed increased activity, leading to a good ligand efficiency ($IC_{50}=3.2 \mu\text{M}$, $LE=0.39 \mu\text{M}$). The inhibitors were modified systematically and a structure–activity relationship was derived.



We further analyzed the fragments regarding binding site and mode of action. Thereby the compounds turned out to be irreversible inhibitors. Competition experiments by SPR verified the same binding site as the natural substrate ACoA. In *P. aeruginosa* PA14, the inhibitors significantly reduced extracellular HHQ and PQS levels, validating PqsD as a target for the development of anti-infectives.

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P540

Cooperative Target/Phenotype-Based Drug Discovery Approach for the Development of Novel Small-Molecule Inhibitors Targeting Receptor Tyrosine Kinase-Associated Cancer Genotypes

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New classes of therapies targeting specific proteins perturbed in cancers have been heralded as “smart drugs” that more effectively target the disease than current radiotherapy and chemotherapeutic regimes. However, disappointing results in recent clinical trials indicate that a major challenge to the development of successful targeted therapies for treating cancer is overcoming heterogeneity

in target mechanism among patients and inherent or acquired drug resistance. Most drug discovery programs begin with a screening campaign for inhibitors against a single protein target. Subsequent chemical and target selectivity optimization is typically based upon “on-target” potency. Consequently, current cancer drug discovery approaches are not appropriately tailored to the complex mechanisms which exist within the disease.

Using an innovative drug discovery approach that merges the field of dynamic template-assisted lead discovery^[1] with image-based multiparametric phenotypic screening,^[2] a library of small-molecule inhibitors has been developed to target receptor tyrosine kinases shown to be important in certain cancers. Drug design was based on the target’s natural ligand, current commercial drugs and the crystallised structure of the target. The compounds were designed to yield initial structure requirements of the target and to be water soluble. Multiparametric high-content image-based phenotypic screening assays were developed and optimized for in vitro studies. Primary phenotypic end points included cell viability, cell cycle, apoptosis and invasion using both target-expressing and non-target-expressing cancer cell lines. Investigation using these cell-based phenotypic models gave preliminary data along with providing structure–activity relationships, which led to the discovery of novel kinase inhibitors against the target. This initial data coupled with information from the crystal structure of the target, is to be used to compile a second generation of compounds aimed at improving both potency and target specificity.

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P541

Immunomodulatory Properties of Nod2-Agonistic Desmuramyldipeptides

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There is a pressing need for the development of novel adjuvants for human use. The minimal bioactive structure of bacterial peptidoglycan (PGN), muramyl dipeptide (MDP) and its derivative murabutide (MB), have long been known for their adjuvant activities. For this reason, a series of novel desmuramyldipeptides have been designed and synthesized as part of our search for therapeutically useful MDP analogues. Since nucleotide oligomerization domain 2 (Nod2) is a putative receptor for MDP, we used engineered HEK293 cells overexpressing Nod2 to screen and validate our compounds for their Nod2-agonist activity. Their immunomodulatory properties were subsequently assessed in vitro, by evaluating their effect on proin-

flammatory cytokine production of phorbol 12-myristate 13-acetate (PMA)/ionomycin-stimulated human peripheral blood mononuclear cells (PBMC). Herein, we present novel desmuramyl dipeptides, the most active of them possessing immuno-enhancing properties as a result of their potent Nod2-agonistic effect.

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Synthesis, NO-Donor Ability, Analgesic and Anti-platelet Activity of New Furoxanyl Hybrid Compounds

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Introduction: Nitric oxide (NO) plays a fundamental role in keeping blood vessels in a dilated state and in maintaining the surface of endothelium nonthrombogenic. NO stimulates the soluble guanylate cyclase (sGC) and increased cGMP levels leading to vasodilatation and inhibiting platelet aggregation. Furoxan (1,2,5-oxadiazole *N*-oxide) have been explored in medicinal chemistry with vasodilating, antiplatelet and analgesic properties. Several reports have been demonstrated that the capacity to generate nitric oxide by furoxan derivatives is responsible for its antiplatelet activity.^[1] Several reports in literature have demonstrated the importance of the *N*-acyl hydrazone subunit for compounds with antiplatelet and analgesic activities.^[2] So, in this work using molecular hybridization non-steroidal anti-inflammatory drugs (NSAIDs) was condensed with furoxan derivatives using as spacer an *N*-acyl-hydrazone subunit to obtain 10 new molecules. NO donor ability, analgesic and antiplatelet activity was performed in order to evaluate the furoxanyl hybrid compounds (**1a,b–5a,b**).

Methodology: 1. *Synthesis.* In the first step, the NSAIDs were totally converted to methyl esters in methanol medium catalyzed by acid. The ester function was converted by nucleophilic substitution to hydrazide function using hydrazine hydrate, 64%. Finally, the hydrazides were reacted with furoxan containing aldehyde function to obtain *N*-acyl hydrazone derivatives. 2. *Detection of nitrite.* A solution of the appropriate compound (20 μ L) in DMSO was added to a mixture (2 mL) of phosphate buffer (50 mM, pH 7.4) and methanol (1:1, v/v), containing L-cysteine (5 mM). The final concentration of the compound was 10–4 M. After 1 h at 37 °C, 1 mL of the reaction mixture was treated with Griess reagent (250 μ L). After 10 min at RT, the absorbance was measured at 540 nm using a spectrophotometer. Standard sodium nitrite solutions (10–80 nmol/mL) were used to construct the calibration curve. The yields of nitrite are expressed as % NO²⁻ (mol/mol). 3. *Antinociceptive activity.* Analgesic activity was

determined in vivo with the acetic acid-induced (0.6%, 0.1 mL/10 g) abdominal constriction test in mice. Swiss mice of both sexes (18–23 g) were used. The compounds were administered orally (100 μ mol/kg) as a suspension in 5% arabic gum in saline (vehicle). Dypirone (100 μ mol/kg) was used as the standard drug. Acetic acid solution was administered i.p. 1 h after the administration of the compounds. Ten minutes after the i.p. acetic acid injection, the number of constrictions per animal was recorded for 20 min. The control animals received an equal volume of vehicle. Antinociceptive activity was expressed as percentage inhibition of the constrictions compared with those in the vehicle-treated control group. The data were analyzed statistically with Student's *t* test at a significance level of *P*<0.05. 4. *Anti-platelet activity.* Blood was withdrawn from rat central artery and mixed with 3.8% trisodium citrate (9:1, v/v). Platelet-rich plasma (PRP) was prepared by centrifugation at 375 \times g for 10 min at RT. The platelet-poor plasma (PPP) was prepared by centrifugation of the pellet at 1800 \times g for 10 min at RT. Platelet aggregation was monitored by the turbidimetric method of Born and Cross using a Chrono-Log aggregometer. PRP (300 μ L) was incubated at 37 °C for 1 min with continuous stirring at 900 rpm. Platelet aggregation was induced by ADP (5 μ M), collagen suspension (5 μ g mL⁻¹), thrombin (2 nM) or arachidonic acid (AA, 100 μ M). Compound (150 μ M) or vehicle DMSO (0.5% v/v) was added to the PRP samples 1 min before addition of the aggregating agent. Acetylsalicylic acid (AAS, 150 μ M), a classical PGHS inhibitor, was used as positive control.

Results: 1. *Synthesis.* The compounds were obtained in three synthetic steps with global yield variable between 42–63%. All compounds were characterized by ¹H and ¹³C NMR spectroscopy, IR spectroscopy and elemental analysis. 2. *Detection of nitrite.* All compounds demonstrated ability to induce nitrite formation between 0.2–14.7%. Compounds **2b**, **3b** and **5b** present higher percentage of nitrite generation than isosorbide dinitrate used as control. 3. *Analgesic activity.* All compounds demonstrated inhibition of abdominal constriction between 21–69%. All compounds, except **2a**, **3b** and **5a**, present analgesic activity higher than dypirone used as control. Compounds **1a** and **4a** presented protection of writhing higher than 60%. 4. *Antiplatelet activity.* We selected for this assay compounds **1a,b** for evaluation of the antiplatelet activity. In the platelet aggregation induced by ADP assay, the compounds showed less aggregation than aspirin e ADP used as control.

Conclusions: The hybrid compounds were obtained with excellent yields and showed NO-donor ability, analgesic and antiplatelet activity. All compounds represent a new analgesic and antiplatelet drug candidates.

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P543

Investigation of Noncanonical Amino Acids and Their Incorporation in Protein

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Noncanonical amino acids are well established as competitive substrates for many aminoacyl tRNA synthetases.^[1] This is particularly evident for the naturally-occurring tyrosine analogue L-3,4-dihydroxyphenylalanine (DOPA), which has been incorporated in proteins produced in an *E. coli* cell-free expression system.^[2] We have recently published an article detailing the incorporation of chlorinated analogues of valine, leucine and isoleucine using the same system.^[3]

The plant-derived amino acids β -N-methylamino-L-alanine (BMAA) and canavanine have been proposed as competitive substrates for glutamate and arginine, respectively, with linked to autoimmune disorders including systemic lupus erythematosus, possibly through incorporation producing immunogenic proteins. Of particular interest is the incorporation of amino acids that have been subjected to oxidative damage, of which there are many examples.^[4] In particular, 3-chlorotyrosine has emerged as an important biomarker for myeloperoxidase-specific oxidation in atherogenesis.^[5] It is likely that upon digestion of oxidatively damaged proteins, some of the resultant noncanonical amino acids are re-incorporated into new protein as part of the aging process.

We sought to analyse the fidelity of the *E. coli* tyrosyl and phenylalanyl tRNA synthetases by investigating tyrosine and phenylalanine analogues with various substituents on the aryl side chain. Substituents investigated were generally small groups such as methyl and fluoro, ranging to groups as large as a nitro. The results of competition experiments using *E. coli* cell extracts to produce peptidyl-prolyl isomerase B (PPIB) incorporating noncanonical amino acids will be presented.

The tyrosyl tRNA synthetases from the human cytosol, *Pyrococcus horikoshii* and *E. coli* were prepared recombinantly in order to analyse the turnover rate of various tyrosine analogues by these enzymes. The crystal structures are known for these three enzymes and will allow us to rationalise differences in fidelity between enzymes by differences in secondary structure.

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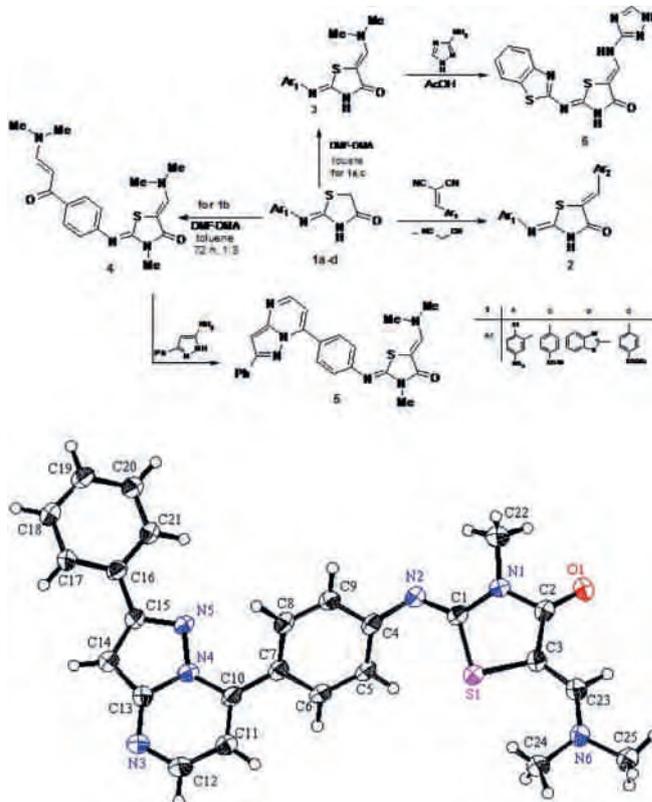
P544

4-Thiazolidinones in Heterocyclic Synthesis: Synthesis of Novel Enaminones, Azolopyrimidines and 2-Arylimino-5-arylidene-4-thiazolidinones

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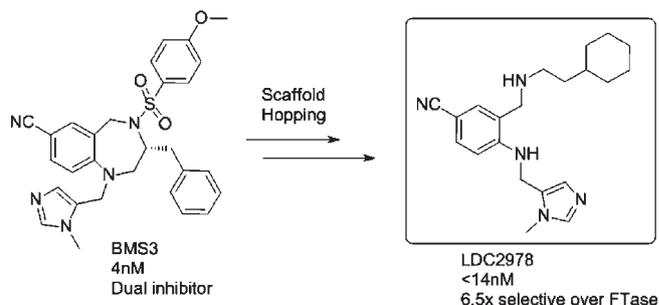
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4-Thiazolidinones **1a–d** was used as key intermediates for the synthesis of 2-arylimino-5-arylidene-4-thiazolidinones derivatives **2a–p** via nucleophilic addition reactions with the arylidene malononitrile. Moreover, 4-thiazolidinones **1a** and **1c** condensed with DMF–DMA to form the corresponding enamines **3**. Otherwise, 4-thiazolidinone **1b** reacts regioselectively with DMF–DMA to afford the enaminone **4**. The latter reacts with many heterocyclic amines affording polyfunctionally substituted fused pyrimidine derivatives, for example pyrazolopyrimidine **5**. Enamine **2c** was also reacted with 3-amino-1,2,4-triazole to afford acyclic product **6**, which could not be further cyclized to the corresponding tricyclic system. The X-ray single crystal technique was employed in this study for structure elucidation and *Z/E* potential isomerism configuration determination. The X-ray crystallographic analyses of eight products could be obtained, thus establishing with certainty the structures proposed in this work.



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P545

Structure-Based Design of Novel, Potent and Selective Inhibitors of Rab Geranylgeranyl Transferase

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Rab geranylgeranyl transferase (RabGGTase) is one of three related enzymes [RabGGTase, farnesyltransferase (FTase) and geranylgeranyl transferase-I (GGTase I)] responsible for post-translational isoprenylation and specifically catalyzes the transfer of geranylgeranyl moieties to Rab proteins. This prenylation is essential for membrane targeting and function of Rab proteins. Experiments with RNAi knockdown and dual FTase/RabGGTase inhibitors, initially developed by BMS as FTase inhibitors, indicate that knockdown/inhibition of RabGGTase induces increased apoptosis.

The observation that Rab proteins are overexpressed in various tumors supports cancer as potential indication of RabGGTase inhibitors, but osteoporosis and infectious diseases have also been discussed.

Here we present a novel series of potent, nonpeptidic inhibitors of RabGGTase with a high degree of specificity over FTase and GGTase. We started from the crystal structure of a nonselective inhibitor (BMS3) using a scaffold-hopping strategy to design a chemically more accessible scaffold, which was subsequently optimized into a nanomolar RabGGTase inhibitor. Structural information regarding the differences in the active site between FTase and RabGGTase were employed to design inhibitors highly specific for RabGGTase. Crystal structures of the ternary complex with the substrates farnesylfarnesylpyrophosphate and geranylgeranylpyrophosphate which confirm the different modes of binding to both enzymes are presented and discussed in the context of the SAR.

P546

Discovery of Phosphatidylinositide 3-Kinases (PI3K) p110 β Isoform Inhibitors as Anti-thrombotic Agents through Structure-Based Fragment Evolution

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Structure-based evolution of the original fragment hits resulted in the identification of (S)-**21**, a novel, potent, selective phosphatidylinositide 3-kinase (PI3K) p110 β isoform inhibitor with favourable in vivo antithrombotic effect-bleeding separation and no insulin resistance. The lecture will summarise tactics and results from the initial fragment-based virtual screening, the structure-based hypotheses to improve potency and PI3K isoform selectivity during fragment expansion and the medicinal chemistry paradigms employed throughout the program. Special emphasis will be placed on the use of in silico, in vitro and in vivo data to support and characterise the various design sets.

P547

Synthesis and Biological Activity of New 5-*O*-Benzyl-7-thiazolyl Isosteres of Goniofufurone and 7-*epi*-Goniofufurone

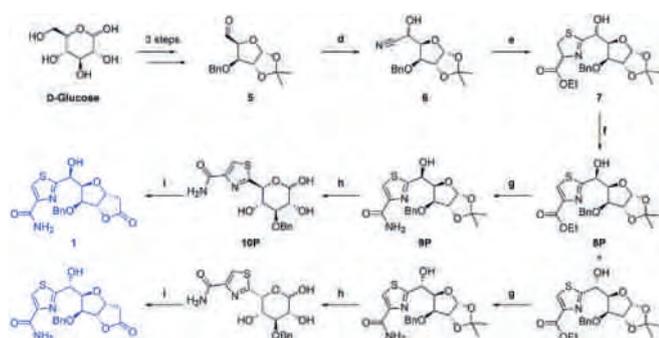
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(+)-Goniofufurone is the naturally occurring cytotoxic styryl lactone isolated from the stem bark of *Goniothalamus giganteus* (Annonaceae) that possesses structure marked by furano-furone bicyclic core.^[1] Herein we report a total synthesis of two novel goniofufurone and 7-*epi*-goniofufurone isosteres bearing a 2-thiazolyl-4-amide moiety instead of the phenyl group at C-7, as well as *O*-benzyl instead of hydroxyl group at C-5 (**1** and **2**, Scheme 1).



Scheme 1. Reagents and conditions: d) TMSCN, Ph₃PMel, CH₂Cl₂, RT; e) L-Cysteine ethyl ester hydrochloride, MeOH, Et₃N, RT; f) CBrCl₃, DBU, CH₂Cl₂, 0°C→+4°C; g) MeOH/NH₃, RT; h) 90% aq TFA, 0°C; i) Meldrum's acid, DMF, Et₃N, 46–50°C.

The key intermediate (**6**) was obtained by addition of trimethylsilyl cyanide to known aldehyde **5**, which was readily available from D-glucose through a modified literature procedure.^[2] Thiazole ring was introduced in two consecutive steps (e, f), which included cyclization and oxidation, respectively. After the ammonolysis (g), which provided amido group at C-4', hydroxyl groups at positions C-1 and C-2 were deprotected (h). After the γ -lactone function was built (i), final products **1** and **2** were obtained. Antiproliferative activity of both analogues against a number of tumor cell lines will be presented.

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P548

Versatile Synthesis and Biological Evaluation of C-5'' and C-6''-Modified α -GalCer Analogues as New iNKT Cell Ligands

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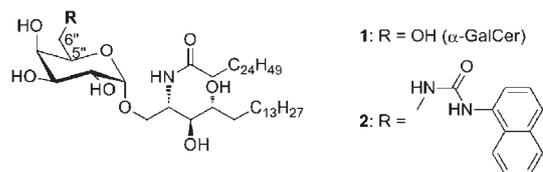
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α -Galactosylceramide (**1**) is known as the prototypical antigen for iNKT cells. Following complexation with the antigen presenting glycoprotein CD1d, α -GalCer is recognized by iNKT cells, which, after activation, produce a rapid burst of Th1 and Th2 cytokines. The fact that both kinds of cytokines antagonize each other's effect is seen as a considerable drawback for therapeutic applications. Hence, much research efforts are directed towards the identification of α -GalCer analogues capable of skewing the iNKT cell responses towards a more biased Th1 or Th2 profile.

Based on the interesting Th1-biased *in vivo* response induced by analogue **2** and the recently obtained crystal structure of this compound complexed to mCD1d and the TCR,^[1] we synthesized a diverse set of derivatives modified at the C-5'' or C-6''-position of the galactopyranosyl ring.



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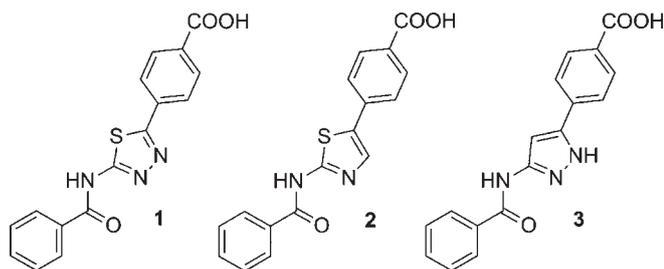
Binding Energy Estimation of CK2 Inhibitors by the Ab Initio-Based Fragment Molecular Orbital Method

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Protein kinase 2 (CK2) is one of the serine/threonine kinases and plays a crucial role in the progression of immunogenic renal injury. We have found a potent inhibitor with the phenyl-thiadiazole scaffold, compound **1** ($IC_{50}=4.4 \mu\text{M}$), by means of in silico screening followed by derivative synthesis. Based on the CK2 complex structure with compound **1**, phenyl thiazole and phenyl pyrazole derivatives **2** and **3**, respectively, were designed, and their binding energies are estimated by the ab initio-based fragment molecular orbital (FMO) method by the following calculation scheme: First, the binding geometry was optimized by the FMO-MP2/6-31G basis set using a truncated complex model which includes amino acid residues within 8 Å from the ligand molecule. Then, the binding energy of the ligand in aqueous medium was calculated using the refined whole complex structure by the FMO-PCM/6-31G* basis set, considering the electron correlation energy (MP2 level) in the gas phase. As the estimated binding energies indicated, more than 100-fold inhibitory potencies could be expected for compound **2** and **3**. Both compounds were synthesized, and the inhibitory activities were measured. Compound **2** actually showed just 100-fold potency compared with compound **1**; however, compound **3** was less potent than compound **2**.

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Thiazolopyridone Derivatives: A Novel Family of Positive Allosteric Modulators of mGlu5 Receptor

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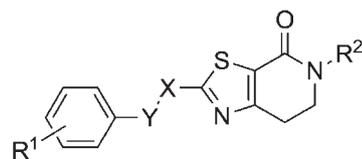
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In light of the NMDA receptor hypofunction hypothesis of schizophrenia,^[1] metabotropic glutamate 5 (mGlu5) receptor activation has emerged as one of the most appealing nondopamine based approaches proposed and investigated in recent years for potential therapeutic intervention of schizophrenia.^[2] As the development of orthosteric agonists for mGlu5 receptors (as well as for the other mGlu receptors) may be hindered by multiple challenges, (e.g., poor drug-like properties, elusive selectivity or potential tolerance development) current strategies have mainly focused on the identification of positive allosteric modulators (PAMs) instead.^[3] This mGlu5 allosteric approach has yielded its first promising results as activity in various preclinical schizophrenia and cognition animal models has already been reported for different mGlu5 receptor PAMs.^[3]

Starting from an HTS hit, a focused medicinal chemistry optimization has led us to the identification of a series of thiazolopyridone derivatives as a novel class of mGlu5 receptor PAMs. These compounds potentiate receptor responses in recombinant systems and have also proven to be efficacious in preclinical models of psychosis. Evolution of our medicinal chemistry program, SAR and SPR analysis as well as a detailed profile for optimized mGlu5 receptor PAM JNJ42659604 will be described.



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P551

The Discovery of Potent and Selective Malarial DHODH Inhibitor Compounds Based on 4-Aminocoumarin and 4-Aminopyran-2-one Scaffolds

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The emerging resistance to antimalarial drugs of the most fatal species responsible for malaria, *Plasmodium falciparum* (Pf), has driven the search for antimalarials that affects novel drug targets in the parasite.^[1] So far, there is no antimalarial drug on the market that is designed to target the enzyme dihydroorotate dehydrogenase (DHODH). DHODH is a well-characterised and promising drug target that catalyzes the rate-limiting step in de novo pyrimidine synthesis. Pyrimidine biosynthesis is particularly important for the survival of the malaria parasites because, unlike humans, they lack the ability to salvage pyrimidine.^[2]

In our search for new PfDHODH inhibitors as antimalarials, in silico design was performed to find new potent compound classes. The design focused on polar interactions with two key residues in the PfDHODH inhibitor binding site, one arginine and one histidine, as well as hydrophobic interactions in a subsite lacking polar amino acid side chains. Inspired by the proposed binding mode of a coumarin human DHODH inhibitor^[3] and taking into account the structural differences between the binding sites of PfDHODH and human DHODH, new derivatives of 4-aminocoumarins and 4-aminopyran-2-ones were designed as putative inhibitors of PfDHODH. A 4-naphthylamino-coumarin was found to be a sub-micromolar inhibitor against PfDHODH showing greater than 100-fold selectivity compared to human DHODH. In addition, it inhibited the proliferation of *P. falciparum* parasites in culture with an EC₅₀ value of 6.2 μM while showing no detectable human cell toxicity. Another nontoxic compound with selectivity for PfDHODH, a 4-naphthylamino substituted 2-pyranone, was highly potent in the parasite assay with an EC₅₀ value of 0.85 μM.

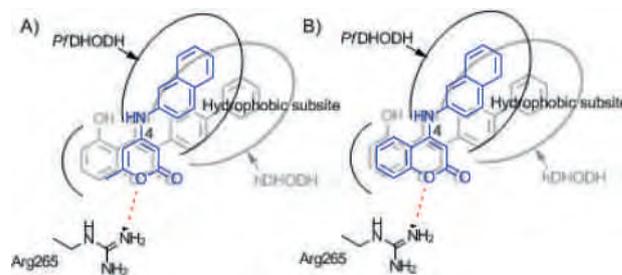


Figure 1. Pharmacophore model of the binding mode for A) the N-substituted 4-amino-pyran-2-ones (blue) and B) the N-substituted 4-aminocoumarins (blue). The superimposed pharmacophore model of the hDHODH inhibitor 3-(biphen-4-yl)-4,5-dihydroxycoumarin in the binding site of hDHODH is shown in gray.

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P552

Rational Design of Orally Active C5aR (C5a Receptor) Allosteric Modulators and In Vivo Efficacy in Inflammatory Pain Models

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Aim: This study aims to evaluate the in vitro selectivity and potency of DF2593A, a novel C5aR allosteric modulator, and its in vivo efficacy in acute and chronic inflammatory models.

Methods: *Molecular modeling:* We previously described Reparixin as a new candidate drug in a novel class of small molecular weight (SMW) noncompetitive inhibitors that binds to CXCR1/2.^[1] Despite the low overall homology between CXCR1 and C5aR, we discovered that the allosteric site of Reparixin on CXCR1/2 is functionally conserved on C5aR. Combining molecular modeling techniques and single-point mutagenesis experiments, we rationally developed a novel class of selective SMW noncompetitive modulators. In particular, DF2593A was selected and characterized to investigate its effect in several experimental models of inflammatory hypernociception. *In vitro assays:* The functional activity of the molecule was carried out by using the C5a-induced PMN chemotaxis. Migration of human and mouse PMN or human

monocytes was evaluated by a microchamber technique in a 48-well microchemotaxis chamber. *In vivo model*: The efficacy of DF2593A in preventing mechanical hypernociception was firstly investigated in different inflammatory pain models, particularly in the zymosan and complete Freund's adjuvant (CFA) models in mouse.^[2] Six hours after zymosan injection in the femoral-tibial joint (150 mg/joint), DF2593A was orally administered and hypernociception was evaluated hourly until 24 h. In the CFA model, mechanical hyperalgesia was measured and DF2593A was given 24 h after CFA. Mechanical hyperalgesia was evaluated daily thereafter until 21 days. For both models, mechanical threshold (g) in response to graded mechanical stimulation was measured using up-down method with von Frey filaments.

Results: *In vitro*: DF2593A potently inhibited C5a-induced human and mouse PMN migration (IC_{50} =8.0±0.1 nM and IC_{50} =3.0±0.6 nM). Similar results were obtained with rat PMN. A panel of relevant GPCRs was identified for a counter assay selectivity screening. No significant effect on radioligand displacement and functional assays at 10 μM was observed towards adrenergic α1, α2, β1, β2; histaminergic H1, H2; cannabinoid CB1, CB2; dopaminergic D2, D3; opioids (μ, δ, κ, ORL₁) and neurokinin NK1. Furthermore, at the same concentration (10 μM), no effects were found in the TRPV1, TRPM8, TRPV4 and TRPA1 ion channels. *In vivo*: Oral administration of DF2593A (1 mg/kg) greatly attenuated mechanical allodynia at 24 h post-dose in the zymosan model. At the tested dose, DF2593A completely abolished (90% inhibition) neutrophil migration in the joints. Similarly, the oral administration of DF2593A 24 h after CFA injection (and then daily until 21 days), greatly attenuated the mechanical threshold (g) when compared with the saline treated group.

Conclusions: In the present study, we firstly identified a novel and selective C5a allosteric modulator, DF2593A, that potently inhibited C5a-induced human and mouse PMN migration *in vitro*. On the basis of these data, we investigated the effects of the selected DF2593A in several acute and chronic inflammatory pain models, like zymosan and CFA models. DF2593A was able to provide relief in mechanical allodynia in both the investigated models. These findings enforces the hypothesis that C5aR blockade may be a novel potential approach in reversing enhanced mechanical sensitivity in inflammatory pain conditions and suggest C5aR as an innovative target for the therapy of chronic pain.

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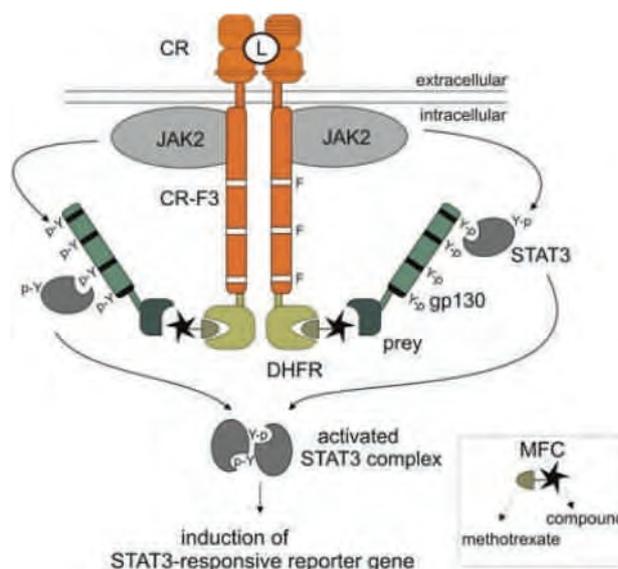
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P553

MASPIT, a High-Throughput Assay for the Identification of Cytosolic Targets of Small Molecules: Proof of Principle

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The identification of all cellular targets of biologically active small molecules not only plays a pivotal role in the development of new drugs but also provides information for the potential use of known drugs for the treatment of other medical conditions through the modulation of new targets. MASPIT,^[1] a three-hybrid technique based on the cytokine receptors JAK/STAT system allows rapid intracellular screening for targets of small molecules against a large collection of specifically modified humane cytosolic proteins with simple photometric readout. To this end, a multifunctional methotrexate reagent was designed and synthesized, which in turn allowed for validation of the MASPIT assay FK506 as the pilot compound.

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P554

Dual PI3K/ERK Inhibitor AEZS-136, a Pyrido[2,3-*b*]pyrazine Derivative with Potent Antitumour Activity under Preclinical Development

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Dysregulated signalling pathways have been implicated to promote cancer cell survival and growth. RAF/MEK/ERK and the PI3K/Akt cascades are among the best characterized cancer-related signalling pathways. The use of dual pathway inhibitors may provide advantages over single pathway inhibitors or combination therapy. Specifically, simultaneous blockage of the RAF/MEK/ERK and the PI3K/Akt pathways may result in enhanced antitumour potency and improved drug tolerability. Further, in comparison to combination therapy, a dual inhibitor may provide reduced toxicity and improved patient compliance.

Here, we present AEZS-136, a unique orally available dual PI3K/ERK inhibitor. AEZS-136 was identified during a medicinal chemistry program to optimize derivatives of the pyrido[2,3-*b*]pyrazine structure class. Presented are the synthesis, pharmacological activity in the nanomolar range (i.e., ERK1/2: IC₅₀ ~50 nM and PI3K: IC₅₀ ~100 nM), physicochemical and ADME data of AEZS-136, together with promising in vivo data. Encouraged by the promising in vitro, ADME and in vivo profiles, AEZS-136 is under development for clinical phase I trials.

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One-Pot Synthesis of Novel 2-Arylpyrrolo[2,3,4-*k*]acridin-1(2*H*)-ones

Fattaneh Narchin, Hassan Kefayati

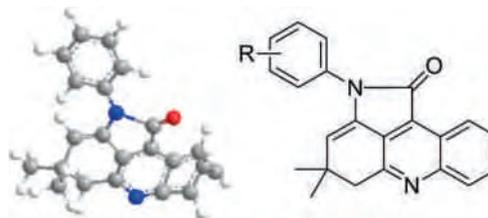
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Pyrroloacridines and pyrroloacridones are of particular interest because they have a variety of interesting biological activities. Significantly, members of this family are active in assays for antihelminthic,^[1] antitumor,^[1,2] antifungal,^[3] and DNA binding.^[4-6] These abilities are specifically important in inhibiting the growth of cancerous cells, making these compounds ideal for developing novel anticancer drugs.

Plakinidines and alpinkidine are pyrroloacridines that have been obtained from marine sources.^[1,7-10] Only a few reports are available for the synthesis of pyrroloacridines and therefore the synthetic versatility of these compounds needs to be explored.

As a result of their significant potential as therapeutics, a considerable synthetic attention has been directed at the development of efficient methods toward the construction of pyrroloacridine

moiety. So, in this research, we wish to introduce a new method for the synthesis of 4,5-dihydro-4,4-dimethyl-2-arylpyrrolo[2,3,4-*k*]acridin-1(2*H*)-one as a new class of pyrroloacridine (R=H, Cl, I, CH₃, NO₂, OCH₃).



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P556

Design, Synthesis and Evaluation of Activity-Based Probes for Urokinase Plasminogen Activator (uPA)

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Urokinase-type plasminogen activator (uPA), a trypsin-like serine protease, plays a key role in extracellular proteolytic events associated with tumor cell growth, migration, angiogenesis and metastasis. In breast cancer, high levels of uPA and/or its endogenous inhibitor PAI-I are correlated with poor patient outcome and tumor aggressiveness. Moreover, for breast cancer, the highest level of evidence (LOE) for grading clinical utility of tumor markers was achieved.

Quantification of uPA and PAI-1 antigen levels is routinely performed by commercially available ELISA kits. However these tests do not discriminate between active and inactive uPA and patient tumor tissue is required. Considering the high prognostic value of uPA and the shortcomings of the ELISA test, it would be useful to have a tool, which could visualize or capture uPA catalytic activity on a qualitative and quantitative manner in *in vitro* and *in vivo* settings.

Up to now, at least two uPA visualizing probes are reported in the literature but they are characterized by disadvantages such as a high molecular weight and a peptide-derived structure. Both probes are so-called internally quenched substrates of uPA that will release a fluorochrome-bearing fragment upon proteolytic cleavage. An alternative and probably more advanced approach to label/visualize catalytic active enzymes is the use of selective activity-based probes. These kinds of probes are designed to react in a covalent manner with the active site of active proteases, in this way tightly linking the fluorescent reporter group directly to the target enzyme.

The poster presents the design, synthesis and evaluation of the first uPA selective nonpeptidic activity-based probe bearing a fluorescent group (rhodamine), which allows *ex vivo* and *in vivo* imaging of tumors in an orthotopic breast cancer model in rodents.

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P557

Potent and Selective TRPM8 Antagonists Ameliorate Mechanical and Cold Allodynia in a Rat Model of Neuropathic Pain

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Aim: This study aims to evaluate the *in vitro* selectivity and potency of DFL23212 and DFL23448, two novel TRPM8 ion channel antagonists, and their *in vivo* effects in the chronic construction injury (CCI) model in rats.^[1]

Methods: *Molecular modeling:* After the HTS feasibility phase, two chemotypes were identified. Molecular modeling studies were performed on the 3D homology model of hTRPM8 previously characterized by Pedretti et al.,^[2] the only TRPM8 model known and published

of the full tetramer. The putative binding for both chemotypes was validated by extensive single-point mutagenesis experiments and Shild analysis. *In vitro assays:* The functional activity of the molecules was initially determined in HEK-293 cells stably transfected with the human TRPM8 gene. The TRPM8 cell line was analyzed for the response to various compounds using a Ca²⁺ mobilization-dependent fluorescence signal in 96 MTP format. Then, the activity of the compounds was tested and confirmed by patch clamp and temperature functional assay on the same cell line. *In vivo model:* In male rats, neuropathic pain behavior was induced by ligation of the sciatic nerve according to the method described by Bennett and Xie. Under inhalation anesthesia, the left hind paw sciatic nerve was exposed at mid-thigh level, and four ligatures were loosely tied around the nerve. On days 7 and 14, mechanical and cold allodynia were assessed. Paw withdrawal threshold (PWT, g) in response to graded mechanical stimulation was measured using up–down method with von Frey filaments 1 h and 3 h after intravenous treatment with test compounds. Cold sensitivity was assessed (1 h and 3 h post-dose) with acetone (15–20°C), applied to the dorsal surface of the ligated paw. Basal responses were measured on the days before treatment.

Results: *In vitro:* DFL23212 and DFL23448 showed an activity on the calcium mobilization induced by cooling agent (IC₅₀=0.2 nM and IC₅₀=30 nM, respectively) and on the cold stimulation (IC₅₀=3 nM and IC₅₀=28 nM, respectively) induced by a temperature ramp from 25°C to 14°C. Similar results were obtained in response to other TRPM8 agonists, like icilin, menthol and WS-12. In the patch clamp assay, DFL23212 and DFL23448 also showed biological activity (IC₅₀=25 nM and IC₅₀=55 nM, respectively) towards TRPM8. A panel of relevant GPCRs was identified for a preliminary selectivity screening. No significant effect on radioligand displacement at 10 μM was observed on muscarinic M2, M3; adrenergic α1, α2, β1, β2; histaminergic H1, H2; cannabinoid CB1, CB2; dopaminergic D2, D3; opioids (μ, δ, κ, ORL₁) and neurokinin NK1. Furthermore, at the same concentration (10 μM), no effects were found in the TRPV1, TRPV4 and TRPA1 ion channels. *In vivo:* Intravenous administration of DFL23212 and DFL23448 at 10 mg/kg significantly attenuated mechanical and cold allodynia at 1 h and 3 h post-dose at 7 and 14 days after injury.

Conclusions: In the present study, we firstly identified two novel molecules that act as potent and selective TRPM8 antagonists *in vitro*. Thereafter, we investigated the effects of the selected TRPM8 antagonists in the rat CCI-induced neuropathic pain model and observed that the compounds were clearly able to provide relief in mechanical and cold allodynia following nerve injury. These findings support the hypothesis that TRPM8 blockade may be a novel potential approach in reversing enhanced mechanical sensitivity in neuropathic pain conditions and suggest TRPM8 as a novel target for the therapy of chronic pain.

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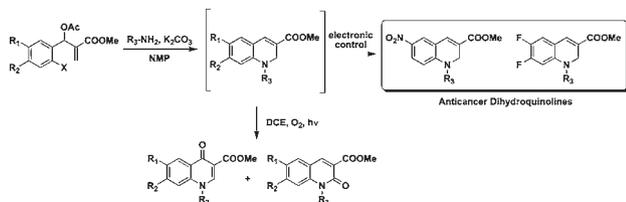
Synthesis of Therapeutically Important Quinolones and Dihydroquinolines from Baylis–Hillman Acetates

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Quinolones, exemplified by ciprofloxacin, levofloxacin and norfloxacin, are well-known antibacterial agents, which exert their effect by targeting bacterial topoisomerase IV and DNA gyrase.^[1] Recent studies have shown that such 4-quinolones and the related 2-quinolones also exhibit anti-HIV, anticancer, and antimalarial activities, which shows the wide therapeutic potential of this class of compounds.^[2] Our studies have shown that Baylis–Hillman acetates on reaction with amines undergo tandem $S_N2'-S_NAr$ cyclization to give 1,2-dihydroquinolines, which on exposure to light and oxygen afford 4- and 2-quinolones through sensitized oxidation or a ($\Delta^{3,4}-\Delta^{2,3}$) shift-oxidation cascade.^[3-4] Subsequent investigations revealed that such 1,2-dihydroquinolines can gain stability if the aromatic ring is substituted with an electron-withdrawing group, such as NO_2 . Based on this, a number of stable dihydroquinolines with different N-substitutions were synthesized, and their antiproliferative effects were assessed on HeLa, SiHa and SW480 cancer cell lines. This presentation gives a detailed account of our synthetic efforts towards 4- and 2-quinolones, and stable 1,2-dihydroquinolines. Anticancer activities of dihydroquinolines and their cellular effects showing their ability to induce apoptosis will also be discussed.^[5]



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P559

Design, Synthesis and Evaluation of Small-Molecule Inhibitors for Phospho-histidine Phosphatase

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N-Phosphorylation of histidine in vertebrates is a phenomenon that is generally overlooked, despite the importance of histidine N-phosphorylation in prokaryotes and protein phosphorylation in general.^[1] Few reports have appeared on kinases and phosphatases responsible for phosphorylation/dephosphorylation of histidine residues in eukaryotic proteins. Altogether, only one phospho-histidine-specific enzyme, phospho-histidine phosphatase or PHPT1 (a.k.a, PHP1 or PHP14), has been described so far.^[2,3] Recently, this enzyme has been shown to play a major role in lung cancer metastasis.^[4] siRNA knock-out experiments have identified PHPT1 as a potentially new drug target. In line with our ongoing investigations on eukaryotic N-phosphorylation, we intend to inhibit PHPT1 with small substrate-like molecules mimicking the phospho-histidine dephosphorylation transition state.^[5]

We have developed the first continuous photometric assay that detects PHPT1 activity. Using this assay, we identified and chemically optimized small-molecule inhibitors. Currently, we are in the process of further profiling these compounds in cellular assays.

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P560

Design, Synthesis and Analgesic Activity of New *N*-Acylhydrazone Derivatives Planned as New Compounds to Treat Sickle Cell Disease

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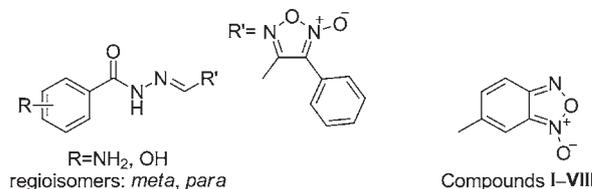
Introduction: Sickle cell disease (SCD) is the hereditary chronic hemolytic anemia most prevalent worldwide.^[1] Despite several reported symptoms, pain is the main complication described by all patients.^[2] Acute pain is the main cause of morbidity and the common cause of hospitalization of SCD patients.^[3] The *N*-acylhydrazone subunit was considered as a single molecular framework and a privileged structure employed to design several new lead compounds with diverse pharmacological activities, including analgesic and anti-inflammatory effects.^[4] In addition, some reports have demonstrated that furoxan and benzofuroxan derivatives could be explored in medicinal chemistry with vaso-dilating, antiplatelet and analgesic properties useful to treat SCD.^[5] In this report, we describe the design, synthesis and pharmacological evaluation of furoxan and benzofuroxan *N*-acylhydrazone derivatives (I–VIII) designed as novel analgesic drug candidates, planned to treat SCD symptoms.

Methodology: In the first synthetic step, the amino or hydroxybenzoic acid derivatives were converted to the corresponding methyl esters in methanol catalyzed by acid. The ester function was then converted by nucleophilic substitution to a hydrazide using hydrazine hydrate (80%). Finally, the hydrazides were reacted with phenylfuroxan or benzofuroxan-containing aldehydes to obtain the *N*-acyl hydrazone derivatives. Analgesic activity was determined in vivo using the acetic-acid-induced (0.6%, 0.1 mL/10 g) abdominal constriction test in mice. Swiss mice of both sexes (18–23 g) were used. The compounds were administered orally (100 μmol/kg) as a suspension in 5% arabic gum in saline (vehicle). Dypirone (100 μmol/kg) was used as the standard drug. Acetic acid solution was administered i.p. one hour after the administration of the compounds. Ten minutes after the i.p. acetic acid injection, the number of constrictions per animal was recorded for 20 min. The control animals received an equal volume of vehicle. Antinociceptive activity was expressed as percentage inhibition of the constrictions compared with those in the vehicle-treated control group. The data were analyzed statistically with Student's *t* test at a significance level of <0.05.

Results: The compounds were obtained in three synthetic steps with global yields of 50–68%. All compounds were characterized by ¹H and ¹³C NMR, IR spectroscopy and elemental analysis. All compounds demonstrated inhibition of abdominal constriction between 23–43%.

Compounds I, III, IV and VII demonstrated analgesic activity higher than dypirone used as a control with protection of writhing higher than 40%.

Conclusions: The *N*-acylhydrazones were obtained in excellent yields and showed analgesic activity in the acetic-acid-induced abdominal constriction test in mice. Compounds I, III, IV and VII represent new lead compounds with analgesic properties useful to treat SCD symptoms.



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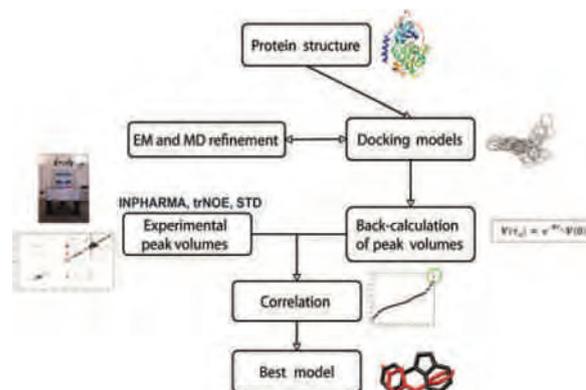
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P561

NMR-Based Ligand–Protein Complex Structure Determination

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Structure-based drug design mainly relies on high-resolution crystal structures of the receptor–ligand complex to obtain the required information for optimizing target binding of small molecules. How-

ever, obtaining crystals and structures of sufficient quality cannot be achieved for approximately 40% of pharmaceutically relevant protein targets. For those target proteins that cannot be crystallized, NMR spectroscopy is an alternative.

We developed a fast and reliable methodology to use experimental NMR data as a scoring function for docking structures: 1) The INPHARMA method^[1,2] derives the relative binding mode of two ligands, given that they target the same binding site; 2) Saturation transfer difference (STD)^[3] yields information on the protein-buried and water-exposed part of the ligand; and 3) Transfer-NOE reveals the bound ligand conformation. For every docking model, the NMR peak volumes of all three data sets are back-calculated with our software SpINPHARMA and then correlated with the experimental data. We show on the example of protein kinase A ligand complexes, that cross validation of docking results against NMR restraints leads to the same binding modes as X-ray crystallography and performs clearly better than docking scoring functions. It is discussed how conformational changes in side chains, crucial for the correct binding mode, can be modelled when molecular dynamics simulations are combined with the NMR data. Furthermore, the methodology is applied on ligand-protein complex structure determination of two interesting ongoing drug targets: 1) The membrane protein G-protein-coupled receptor 40 (FFAR1) for which only homology models exist, the absolute binding mode of fatty acid derivative ligands is revealed; and 2) The tubulin-binding drug epothilone, where an NMR structure competes with an electron crystallography structure.

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P562

Synthesis and Biological Evaluation of Novel Multitarget Hybrid Barbiturates

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Inflammation is believed to be involved in numerous diseases, such as cancer and senile dementia Alzheimer's type. Reactive oxygen species (ROS) are produced during the inflammation process by phagocytic leukocytes that invade the tissue. These ROS are involved in the lipoxygenase (LOX)-mediated conversion of arachidonic acid into proinflammatory intermediates and can induce mutations, cell proliferation, apoptosis, differentiation, and senescence.^[1]

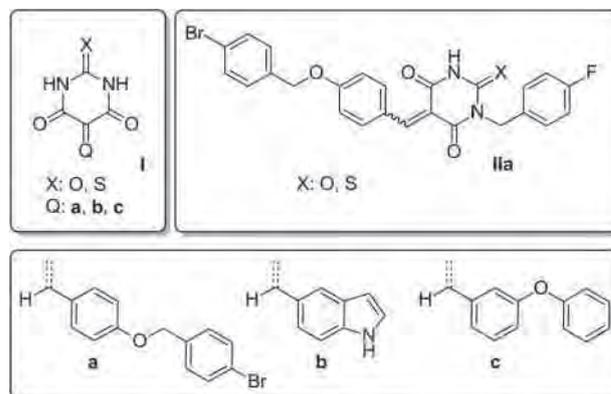
In continuation to our previous research on LOX inhibitors with anticancer activity and acknowledging the biological role of autotaxin /ectonucleotide pyrophosphatase/phosphodiesterase family mem-

ber 2 (ATX or ENPP2) and G-protein-coupled receptors (GPCRs) in cancer, we proceeded to develop, using computer-aided drug design (modelling and QSAR studies), novel multitarget barbiturates. We performed in silico calculations, and an initial base of approximately 110 barbiturate analogues was designed and combined with the extracted results of the QSAR studies. The most prominent of those molecules (measured in Kcal/moles) were selected to be synthesized.

For the synthesis of the selected barbiturate analogues (I), we used known and modified classical methods (Knoevenagel, Diels-Alder, reductions and ring closure reactions), as well as green chemistry, microwave irradiation and/ or sono-chemistry. A de novo approach was followed for the synthesis of mono-substituted ureas and consequently the designed barbiturates.

Knoevenagel condensation reaction in two different synthetic approaches was followed to give the target compounds in 60–75% yield. A microwave-assisted reaction was also applied leading to quantitative yields.^[3] Mono-substituted ureas were synthesized giving mono-substituted barbiturates in 75–85% yield. Structures of the products were confirmed by their melting points, elemental analysis, IR, ¹H and ¹³C NMR spectra.

Compounds were preliminary tested for their ability to inhibit soybean lipoxygenase in vitro (19–71%) and to interact with the stable free radical DPPH (40%).^[4] Our results were compared with appropriate standards. The results are discussed in terms of structural characteristics and physicochemical properties.



The chosen synthetic pathways were successful in terms of yields. The results of our biological tests showed that this group of synthesized compounds could be used as leading compounds for further derivatization in our research for effective antiproliferative and LOX inhibitory agents.

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P563

Synthesis and Osteoclast Formation Inhibition Effect of a Novel *N*-Hydroxycarbamimidoyl Compound

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Novel compound, 5-(4-(*N'*-hydroxycarbamimidoyl)-phenoxy)-*N*-(2-(4-ethylphenyl amino)benzo[d]oxazol-5-yl)-pentanamide (**K7**) was prepared in seven steps starting from 2-aminophenol. Its osteoclast differentiation inhibitory effect was evaluated in vitro. Using mouse bone-marrow-derived macrophages (BMMs), we showed that **K7** suppresses the receptor activator of nuclear factor- κ B (NF- κ B) ligand (RANKL)-induced osteoclast formation in a dose-dependent manner with an IC_{50} value of 1.31 μ M. **K7** was shown to inhibit the expression of osteoclast marker genes, calcitonin receptor (CTR) and cathepsin K (CATK), dendritic cell-specific transmembrane protein (DC-STAMP), ATP6v0d2, α v-integrin and β 3-integrin. Furthermore, **K7** inhibited the bone resorptive activity of osteoclasts. In experiments to elucidate its mechanism of action, **K7** was found to suppress RANKL-induced expression of c-Fos and NFATc1, transcription factors that are essential for osteoclast differentiation. An analysis of a signaling pathway showed that **K7** inhibited RANKL-induced activation of p38, extracellular regulated kinase (ERK) and NF- κ B. Finally, **K7** suppressed lipopolysaccharide-induced osteoclast formation and bone loss in the in vivo mouse experiments, suggesting a potential therapeutic strategy for treating diseases involving bone destruction.

P564

5-HT₆ Receptor Antagonists: Synthesis and Biological Evaluation of Benzoisothiazoles

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The 5-HT₆ receptor is interesting as a novel target for the treatment of central nervous system (CNS)-mediated diseases. The receptor is mainly localized in the CNS, and its exclusive distribution in the brain is related to cognition, obesity, and certain neuropsychiatric disorders and neurodegenerative diseases, including depression, schizophrenia, and Alzheimer's disease. As part of our continuous study on 5-HT₆ receptor antagonists, a series of novel benzoisothiazoles, which have an *N,N*-dimethylformimidamide as an ionizable nitrogen group, were synthesized. The coupling reaction of 3-amino-5-nitrobenzothiazole with *N,N*-dimethylformamide and dimethylcarbonyl chloride afforded the desired imines. Then reduction using SnCl₂/ultrasonic irradiation and reaction for forming of arylsulfonamide were performed. The functional efficacy of each compound was evaluated by measuring the level of cAMP in vitro. Stimulation of HER293 cells stably expressing recombinant human 5-HT₆ receptor by serotonin activates adenylyl cyclase, which catalyzes the synthesis of cAMP. The IC_{50} values of the compounds prepared were 1.02–5.04 μ M.

P565

Fragment-Guided Design of Sub-nanomolar beta-Lactamase Inhibitors Active In Vivo

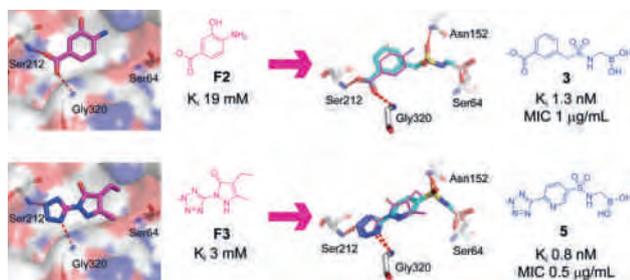
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To overcome compound optimization challenges, fragment-based design was used to guide derivatization of a lead series of β -lactamase inhibitors that had heretofore resisted optimization. X-ray structures of fragments overlaid with the lead structure suggested new functionality and points of attachment. Synthesis of three derivatives improved affinity 20- to 30-fold and improved efficacy in cephalosporin-resistant bacteria. Crystal structures were consistent with the fragment-based design, enabling further optimization to a K_i value of 50 μ M, a 500-fold improvement that required the synthesis of only six derivatives. Compound **5** was further tested in mice. Whereas cefotaxime alone was ineffective in treating mice infected with β -lactamase-expressing *E. coli*, 65% were cleared of infection when treated with a cefotaxime:**5** combination. Fragment complexes offer a path over design hurdles, even for advanced molecules; the series described here may provide leads to overcome β -lactamase-based resistance, a key clinical challenge.

P566

Discovery of a New Class of Highly Potent Inhibitors of Acid Ceramidase: Synthesis and Structure–Activity Relationships (SARS)

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Acid ceramidase (AC) is a ubiquitous cysteine amidase that is located within the lysosome and is responsible for the degradation of the lipid messenger, ceramide.^[1] By regulating ceramide concentration within cells, AC is involved in several disorders associated with deregulation of sphingolipid metabolism. In particular, AC is emerging as an important enzyme in cancer progression and the response to tumor therapy, making its inhibition a promising strategy for cancer treatment. However, current AC inhibitors have limited potency (medium-high micromolar range) and lack drug-like properties.^[2]

Screening a commercial chemical library, we identified the anticancer agent carmofur (5-fluoro-*N*-hexyl-2,4-dioxo-pyrimidine-1-carboxamide) as the first nanomolar AC inhibitor (rat AC, IC_{50} =29 nM) and showed that this compound strongly enhances the antiproliferative effects of two mechanistically distinct antitumor agents, 5-fluorouracil and taxol. These findings suggested that carmofur might be a good starting point for the discovery of new cancer-sensitizing drugs. To explore this possibility, we investigated the uracil scaffold with the aim of exploring structure–activity relationships (SARs) for this class of compounds and discovering more potent AC inhibitors.

The SAR study allowed a first elucidation of the structural features of uracil derivatives that are critical for AC inhibition, as well as the identification of novel double-digit nanomolar inhibitors of AC. The results confirmed that substituted 2,4-dioxo-pyrimidine-1-carboxamides represent a novel class of potent drug-like AC inhibitors. Selected derivatives in this series may provide useful probes to further characterize the functional roles of AC, and assess the therapeutic potential of AC inhibitors, alone or in combination with established treatments, in cancer therapy.

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P567

Mechanism of Enediynes–PBD-Induced Growth Inhibition and Apoptosis in Human Melanoma Cells

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Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are a group of potent, naturally occurring antitumor antibiotics produced by *Streptomyces* species. An enediyne contains either DNA intercalating groups or DNA minor groove binding functions, and these are potent DNA-damaging agents due to their ability to generate benzenoid diradicals. We previously reported an efficient synthesis and antitumor activity of a series of novel PBD hybrids linked with enediynes.^[1] The purpose of this study was to examine the mechanism of the antiproliferative effect of enediynes–PBD agent on human melanoma cells. Cell apoptosis was evaluated by sub-G1 region analysis, caspase-3 colorimetric assay, and M30 CytoDeath staining. Intracellular Ca²⁺ and ROS were measured using Fluo-3AM dye, DCFH-DA probe, and 8-oxoguanine staining. The phosphorylation of MAPKs, ATF-2, and the degradation of PARP were determined by Western blotting. The AP-1 activity was determined by using the luciferase reporter assay. Enediynes–PBD-treated cells resulted in an increased sub-G1 population, caspase-3 activation, PARP cleavage, and more M30 CytoDeath staining. In addition, DC-81-enediyne induced an increase in Ca²⁺ level and ROS generation, which involve p38 phosphorylation and enhanced ATF-2/AP-1 expressions, and ultimately lead to A375 cell apoptosis. In the present study, we highlight a novel enediynes–PBD antitumor proliferation mechanism and suggest this agent has chemotherapeutic activity for melanoma.

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P568

Synthesis of Novel Cyclic Lipopeptides Active against Gram-Negative Bacteria

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The lack of treatment options for multidrug-resistant (MDR) bacteria, such as *E. coli*, *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*, and the emergence of resistance against colistin (one of the last effective

antibiotics against these bacteria), underlines the urgent need for new antibiotics active against serious Gram-negative infections. This is exacerbated by the current lack of drug candidates in the pharmaceutical pipeline.^[1–4] Lipopeptide antibiotics were discovered 50 years ago and consist of a cyclic peptide portion with an attached fatty acid chain, which facilitates insertion into the bacterial membrane.^[5] Members of this class include colistin and the polymyxins as well as the recently approved daptomycin.

Herein, we describe the synthesis of a series of cyclic peptides using solid-phase peptide synthesis (SPPS) with on-resin and solution-phase cyclisation giving access to a number of lipopeptide derivatives. Systematic exchange of individual amino acids and variations in the lipid tail enabled the delineation of some structure–activity correlations. The lipopeptides were evaluated for their antibacterial activity against Gram-negative bacteria and derivatives with good activity against *P. aeruginosa*, *A. baumannii*, and *E. coli* were identified.

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P569

4-Imino-imidazole-2-thiones: Inhibitors of Bacterial Hyaluronidase Obtained by Computer-Assisted Methods and Multi-component Synthesis

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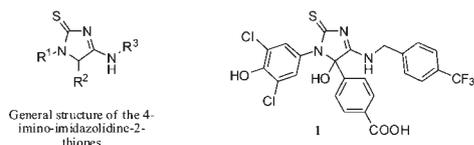
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Enzymes cleaving hyaluronic acid (hyaluronan), the hyaluronidases, are widespread in nature, not only present in the animal kingdom, but also considered virulence factors of microorganism, including several pathogenic strains of *Streptococci*. Weak hyaluronidase inhibitors have been identified among natural products and synthetic molecules.^[1] However, with respect to drug discovery or a rational design of inhibitors, hyaluronidases still represent a class of neglected enzymes. Inhibitors of bacterial hyaluronate lyases could serve as pharmacological tools or even could be used as new drugs against drug-resistant bacteria.

Focusing on a streptococcal hyaluronidase (*Sag* Hyal₄₇₅₅), we report on a target-based approach to lead inhibitors. The combination of innovative computer-assisted methods of drug design and synthesis technologies was applied to identify novel structural motifs. Initially, a compound library comprising 347 inhibitors of the target enzyme was analyzed.^[2] The corresponding structure–activity relationships (SARs) were elucidated and models of the enzyme in complex with potential inhibitors were generated *in silico*. For this purpose, only small molecules predicted to possess drug-like properties and accessible by multicomponent synthesis were taken into consideration.

The suggested compounds were synthesized via parallel synthesis, analyzed and tested for biological activity in medium-throughput. Among 2640 screened samples, 4-imino-imidazolidine-2-thiones were identified as promising “hits” to develop inhibitors of *Sag* Hyal₄₇₅₅. Preparative synthesis and purification of hit compounds revealed inhibitory activities in the micromolar range. However, chemical stability of some derivatives was unsatisfactory, due to autoxidation and decomposition. To cope with this problem, we studied the oxidation process in detail by means of [¹⁸O]-labeling and MS analysis. The results paved the way to the synthesis of more stable molecules.



The substituted 4-imino-5-hydroxy-imidazolidine-2-thione **1** (IC₅₀=8 μM) was identified as one of the most potent inhibitors of *Sag* Hyal₄₇₅₅ known so far. Evaluation of related compounds corroborated the biological data for this class of hyaluronate lyase inhibitors.

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P570

2-Phenylindoles as Inhibitors of Streptococcal Hyaluronidases

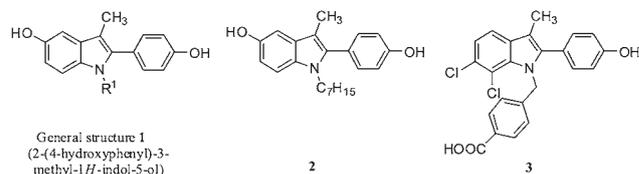
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As shown previously for the bacterial hyaluronidase *Sag* Hyal₄₇₅₅, the introduction of alkyl groups in position 1 of hydroxylated 2-phenylindoles (scaffold: 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol, general structure **1**) led to the discovery of inhibitors with activities in the lower micromolar range. For instance, the *N*-alkylated 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol derivative **2** was identified as an inhibitor with an IC₅₀ value of 22 μM. The potency correlated significantly with the length of the aliphatic chain. Unfortunately, such lipophilic moieties are unfavorable with respect to drug-like properties, for example, due to extremely high plasma protein binding. Furthermore, depending on the *N*-substituent, compounds derived from the 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol scaffold are known for antiestrogenic activity in the low nanomolar range.^[1] Hence, potential binding of hyaluronidase inhibitors bearing a hydroxylated 2-phenylindole motif to the estrogen receptor (ER) should be kept in mind.

In the present study, we describe the synthesis and the pharmacological characterization of a small series of 2-phenylindoles bearing a benzyl substituent in position 1. Compared with the previously described inhibitors of streptococcal lyases, less lipophilic compounds were aimed at to improve the drug-like properties. According to this approach, hyaluronidase inhibitors were obtained with activities in the micromolar range. Modification of the core structure led to potent hyaluronidase inhibitors devoid of cytotoxicity, determined on (anti)estrogen-sensitive MCF-7 breast cancer cells. Analogues bearing chlorine substituents were micromolar inhibitors of the target enzyme. For example, 4-[[6,7-dichloro-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl]benzoic acid (**3**) showed an IC₅₀ value of 6 μM, determined at the pH optimum of *Sag* Hyal₄₇₅₅. Furthermore, compound **3** with an IC₅₀ value of 93 μM proved to be the most potent inhibitor of a related streptococcal enzyme (*Spn* Hyl) known so far.



In summary, the presented compounds can be considered as lead structures for the development of inhibitors of bacterial hyaluronate lyases. Such agents might be useful in combination with antibiotics to combat bacteria producing hyaluronidase as a putative virulence factor.

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P571

Redox Modulation of VLA-4 Activity by the Tellurium Compound SAS Inhibits the Migratory Activity of Murine Melanoma Cells

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Several synthetic tellurium compounds exhibit biological activity. Ammonium trichloro (dioxoethylene-*O,O'*)tellurate (AS101)^[1] is probably the most studied one. It is a potent immunomodulator (both in vitro and in vivo) with a variety of potential therapeutic applications.^[2]

A new inorganic tetra-valent Te^{IV} compound with a novel dimeric structure, octa-*O*-bis-(*R,R*)-tartarate ditellurane (SAS), was recently prepared in our lab. SAS interacts in vitro with thiols and inhibits the activity of human cysteine proteases.^[3] Moreover, SAS is nontoxic and protects the hemopoietic system from chemotherapy-induced damage in mice.

The present study addresses the biochemical mechanism of SAS activity at the molecular level. We demonstrate by FRET that SAS interacts with the VLA-4 (α4β1) integrin, affecting its conformation and inhibiting its activity. In order to explore the functional implications of VLA-4 inhibition by SAS, we conducted several experiments with murine B16 melanoma cells. SAS inhibited attachment of melanoma cells, abundantly expressing the VLA-4 integrin, to its natural ligand fibronectin (FN). Anti-VLA-4 neutralizing antibody inhibited cell attachment to FN, while SAS did not further enhance this suppression. These results suggest that B16 melanoma cells attach FN via VLA-4, being an important target for SAS activity. Moreover, inhibition of cellular attachment by SAS was associated with suppression of matrix metallo proteinases (MMPs) 2 and 9 secretion, known to be regulated by VLA-4, in a dose-dependent manner. Importantly, the consequence of these accumulated activities was in vitro inhibition of melanoma cells migration. Finally, we demonstrated that integrins are possible target of redox inactivation by SAS, by its selective binding to vicinal thiols of cysteine residues within the exofacial domain of the integrins, affecting their biological activity in a reversible manner.



These results highlight the role of the nontoxic tellurium compound SAS in the unique inhibition of the VLA-4 integrin activity in B16 melanoma cells, resulting in suppression of biological processes related to melanoma migration and invasive properties.

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P572

Structure–Activity Relationships in Constrained Benzylglycinamides: A Molecular Template for the Design of State-Dependent Sodium Channel Blockers

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Voltage-gated sodium channel blocking activity is a characteristic of certain anaesthetic, antiarrhythmic, analgesic and antiepileptic drugs. However, many current therapies have mixed modalities, are associated with inconsistent efficacy and are poorly tolerated. Pathological conditions are often associated with rapid channel firing so compounds that slow channel cycling by preferential inactivated state-dependent binding offer the opportunity to mediate disease states while interfering minimally with normal physiology.

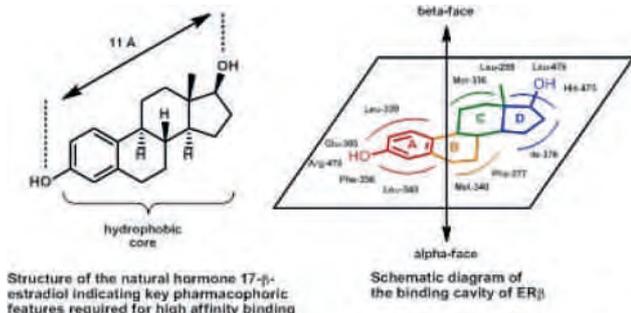
Herein, we describe the discovery and profile of state-dependent sodium channel blockers characterised by an embedded benzylglycinamide moiety. The imposition of specific stereoisomeric and conformational constraints enabled the identification of compounds with enhanced sodium channel selectivities, and modulation of their state-dependent properties. Structure–activity relationships were identified for hERG blockade, and compound classes were discovered with desirable developability characteristics including metabolic stability, brain penetrancy and disease model efficacy.

P573

Design of a Selective Estrogen Receptor Beta Agonist as a Possible Neuroprotective Intervention of Multiple Sclerosis

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The estrogen receptor (ER) is a member of the nuclear receptor superfamily, which functions as hormone-regulated modulators of intracellular signalling and gene expression. ER exists as two subtypes (ER α and ER β), and studies have showed intriguing differences in the tissue distribution and genes regulated by the two subtypes. Emerging data suggest that the development of subtype-selective ligands that specifically target ER β may be a superior approach for the treatment of multiple sclerosis (MS). Current therapies for MS are primarily targeting the immune system exerting anti-inflammatory actions and show effect in the relapse remitting phase when the degree of inflammation is high, but have limited effect in the progressive phase when the degree of neurodegeneration is high. Recently published studies have revealed positive effects of estrogens in experimental autoimmune encephalomyelitis (EAE) that are mediated by both ER α and ER β . By using an ER β agonist for neuroprotective treatment in MS, ER α -mediated side effects, such as increased cancer risks and thrombosis, would be avoided. The high sequence similarity and structural conservation between the ligand binding domains of the two ER subtypes has made it a challenging undertaking to develop subtype-selective ligands for the ERs. The first ER β -selective ligands to be discovered were phyto-estrogens (such as the heteroaromatic genistein) of largely planar topology. The explanation for their modest selectivity is that the binding cavity of ER β is slightly narrower than that of ER α . This allows better packing of flat aromatic ligands such as genistein in the ER β binding cavity compared with ER α . Ligands that enforce strong repulsive interactions with ER α while avoiding corresponding unfavourable interactions with ER β tend to display much greater and more robust selectivity compared with ligands that achieve selectivity through differences in attractive interactions. The current focus of our ER programme is the treatment of multiple sclerosis through the neuroprotective properties of ER β . We will present structural data for a series of highly selective ER β ligands whose selectivity is a result of substituents that are orthogonal to the core producing an overall ligand topology that is non-planar. In addition, we will present data where

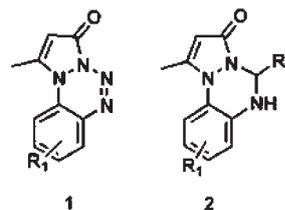
a proprietary selective ER β agonist reduces neurological scorings in a relapsing–remitting EAE rat model and shows neuroprotective effects after glutamate excitotoxicity in vitro, suggesting ER β agonists as a possible novel neuroprotective therapy of multiple sclerosis.

P574

1,2-Dihydropyrazole[1,2-*a*]benzo[1,2,4]-triazine-3-one: Deaza Analogue Tricyclic Scaffold with Valuable Antiproliferative Activity

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1-[1,2-*a*]Benzo[1,2,3,4]tetrazin-3-one derivatives (**1**) have been previously designed as new alkylating agent because of their peculiar chemical behavior. Most of the synthesized derivatives showed antiproliferative activity against more than 50 types of human tumor cell lines reaching in some cases micromolar values.^[1] Here, with the aim of modulating the biological profile, we planned to switch our interest to the deaza tricycle 1,2-dihydropyrazole[1,2-*a*]benzo[1,2,4]triazine-3-one (**2**) exploiting the advantage of introducing new moieties in R2 useful for SAR studies. Various fused pyrazolo-triazine derivatives showed antiproliferative activity as novel potent inhibitors of kinase CK2^[3] and of CYP1A1, the enzyme involved in the metabolism of chemical carcinogens.^[2] Even more, hydrazide derivatives have exhibited remarkable inhibitory activity against SiHa and LS180 human tumor cell lines, with absence of toxicity towards the HSF control cell line.^[4,5] In the present study, we designed and synthesized a set of new derivatives of type **2** containing selected functional groups in order to evaluate their potential anticancer properties.

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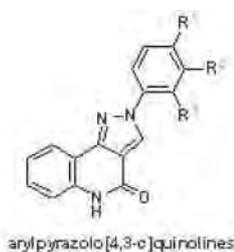
Synthesis of a Novel Pyrazole-Containing Tricyclic Ring System and Its Biological Evaluation as Core of Potential Adenosine A₃ Receptor Antagonists

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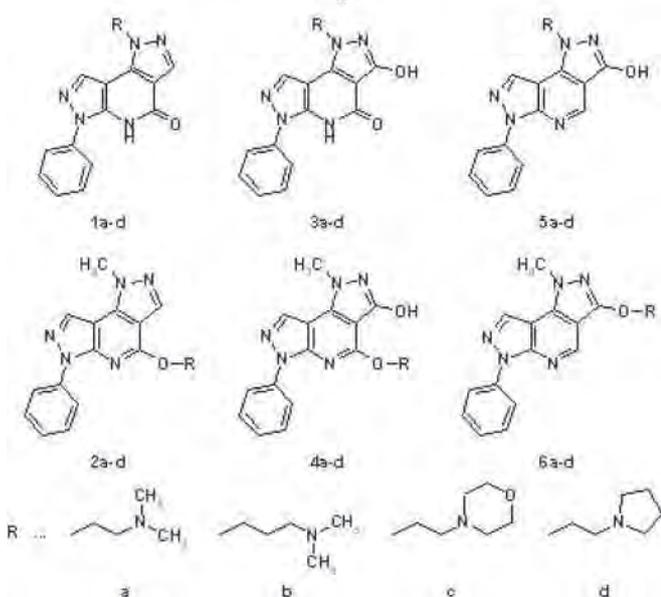
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A



The pyrazolo[3,4-*b*]pyridine system represents an important substructure of a larger number of biologically active chemical entities and is therefore of high interest for medicinal chemists. Moreover, appropriately substituted pyrazolo[3,4-*b*]pyridines can serve as starting materials for the construction of higher anellated systems. In this context, we present the synthesis and functionalization of 6-phenyl-1,6-dihydro-dipyrazolo[3,4-*b*:3',4'-*d*]pyridines of type 1–6. Such compounds can be seen as pyrazole analogues of pyrazolo[4,3-*c*]quinolines—a well-known class of compounds exhibiting a variety of biological activities such as affinity to the benzodiazepine receptor

site (BZR) and the adenosine A₃ receptor; furthermore, the latter show PDE4 inhibition, antiviral and anti-inflammatory activity.^[1] Thus, for instance, Baraldi and co-workers reported adenosine A₃ receptor affinity of type A arylpyrazolo[4,3-*c*]quinolones.^[2] With respect to this finding we prepared a variety of structurally related tricycles 1–6, decorated with appropriate side chains of type a–d. Some of the synthesized compounds showed A₃ adenosine receptor affinity in the nanomolar range as evaluated in radioligand binding assays.

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P576

Method for In Vivo Neurochemical Monitoring: A Tool for Experimental Pharmacology

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In vivo neurochemical monitoring using microdialysis sampling is important in neuroscience because it allows correlation of neurotransmission with behavior, disease state, and drug concentrations in the intact brain. A significant limitation of current practice is that different assays are utilized for measuring each class of neurotransmitter. A high-performance liquid chromatography (HPLC)–tandem mass spectrometry method for monitoring of low-molecular-weight neurotransmitters (dopamine, serotonin, GABA, glutamate) and their metabolites from brain microdialysis samples is the topic of the current work. The method consisted of a 2D-UPLC method, serving the purpose of separating salts from artificial cerebrospinal fluid (ACSF) used in the microdialysis, and a detection method involving LC–ESI-MS, where the selected reaction monitoring mode was used for its extremely high degree of selectivity and the stable-isotope dilution assay for its high precision of quantification. The developed method was characterized by the following parameters: precision was $\leq 12.4\%$ (determined as RSD) for all substrates; mean accuracy was $\leq 11.8\%$ (determined as RE). The method was tested on samples obtained from nucleus accumbens of rat pups after an acute methamphetamine administration. The developed assay could be applied for both a simultaneous analysis of all the four neurotransmitters (dopamine, serotonin, GABA and glutamate) and their principal metabolites in microdialysis samples acquired from the rat brain and the time monitoring of their tenuous concentration changes on picogram level followed by methamphetamine stimulus. The developed assay method is easy to operate, robust and rapid. To the best of our knowledge, it is the first method not using the separation and pre-concentration step and also analyzing such a large number

of neurotransmitters and their metabolites in a single analytical run. The developed method potentially expands the diagnostic/treatment potential in regards to neuropsychiatric and neurological disorders, where the above-mentioned neurotransmitters and their metabolites concentration levels are altered in comparison to normal physiological levels. The method may well contribute to a better understanding of the pathophysiology and pathogenesis of many neuropsychiatric disorders (drug addiction, schizophrenia, Parkinson's disease, Alzheimer's dementia) and in pharmaceutical research of new drugs to treat neurological diseases.

Acknowledgements: The authors wish to acknowledge with gratitude the financial support by the Grant Agency of the Czech Republic (Grant GACR P303/10/0580).

P577

Metabolomic Profiling of Exhaled Breath Condensate in Patients Suffering from Difficult-to-Control Asthma: LC–MS and NMR Metabolomics

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Asthma bronchiale is a chronic respiratory disorder, which is a major public health problem. Because of advances in recent medicine, it can be controlled in most cases with a suitable medication. However, 5–10% of patients still suffer from difficult-to-control asthma (DCA).

Exhaled breath condensate (EBC) is a rich source of a variety of substances, reflecting changes in the environment of the airways. It offers further possibilities for the diagnostics and monitoring of several lung diseases. It is supposed that aerosol particles exhaled in human breath reflect the composition of the bronchoalveolar extracellular lining fluid. Information about the specific biomarkers of lung diseases can be obtained by analysis of EBC.

It is believed that high levels of oxidative stress are key factor in pathogenesis of asthma. We have used metabolomic approach to analyze EBC using liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance (NMR) spectroscopy. The influence of corticosteroid (CS) therapy on the metabolomic profile of EBC in DCA patients was followed. Our hypothesis was that oxidative stress metabolomics in the EBC differs in oral CS-dependent DCA versus DCA treated so far by inhaled CS and compared to control group of healthy volunteers. Concentrations of 22 markers of oxidative stress (malondialdehyde, leukotriens B₄, C₄, D₄, E₄, 8-isoprostane,

5-hydroxymethyluracil, *o*-tyrosine, nitro-tyrosine) were detected by LC–MS. Samples were harvested by standardized protocol (Jaeger EcoScreen). Measured results were analyzed together with FEV₁, eNO₅₀, blood eosinophils and statistically evaluated (ChiSquare, MannWhitney, Kruskal Wallis). Furthermore, a non-targeted metabolomic approach was applied to define changes in the metabolic status of DCA patients.

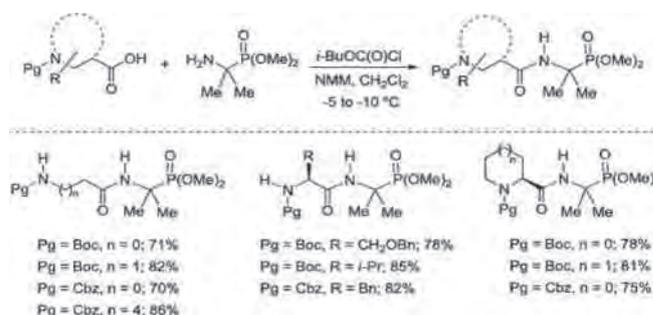
Our preliminary results indicated increased lipoperoxidation and blood eosinophilia from ICS group in comparison to OCS-treated patients. Therefore, we speculate that ICS DCA would benefit from earlier chronic oral CS therapy to prevent irreversible consequences of oxidative stress.

P578

High Efficient Synthesis of Phosphonopeptides Using Isobutyl Chloroformate as a Coupling Agent

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Phosphonopeptides as an important class of modified peptides containing a tetrahedral phosphorus atom have been used as enzyme inhibitors, haptens for production of catalytic antibodies, antibacterial and herbicidal agents.^[1] They contain the phosphonic acid moiety, an important pharmacophore of significant relevance as an isosteric replacement for phosphate or carboxylate functional groups, which are ubiquitous ligands in the active sites of many enzymes.^[2] The bioactivity of phosphonopeptides depends essentially on the stereochemistry of incorporated aminophosphonic acids and amino acids.^[3] Actually, phosphalines are an example of a representative family of antibacterial phosphonopeptides designed to mimic the terminal dipeptide moiety (D-Ala-D-Ala) of bacterial cell wall peptidoglycan acting as peptidoglycan biosynthesis inhibitors in both Gram-negative and Gram-positive bacteria through active transport into bacterial cells by stereospecific enzymes.^[4] Here, we report an efficient synthesis of new phosphonopeptides by coupling amino acids to a quaternary α -aminophosphonate through mixed anhydride intermediate in goods yields.

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P579

New Benzimidazolone Derivatives as Ligands for the 5-HT₇ Receptors

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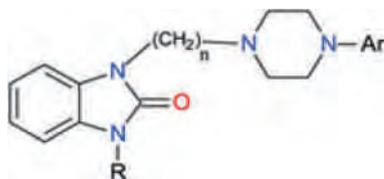
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Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter with multiple physiological functions.^[1] Among the seven subtypes of receptors that mediate 5-HT functions,^[2] the 5-HT₇ receptors are the latest discovered (1993).^[3] Their distribution both in the central nervous system (CNS) and in peripheral tissues is highly associated with their implications in psychiatric disorders, depression, anxiety and mood, learning and memory, epilepsy, inflammatory processes, ileum peristalsis, to cite just a few recent studies on 5-HT₇ receptor complex system.^[4]

Among the many building blocks used for the design of serotonergic ligands, arylpiperazines hold a special place because they incorporate the ideal geometry of two of the essential features necessary for a good affinity: a basic amine, protonated at physiological pH, and aromatic feature. Arylpiperazines, initially discovered as 5-HT_{1A} receptor ligands, also show good affinities for the 5-HT₇ receptor, most probably due to the strong similarities between the binding site of those receptors.



In recent years, our research group has been involved in the development of new benzimidazolone derivatives able to selectively bind to the 5-HT₇ receptor over the 5-HT_{1A} receptor.^[5] In the present communication, we report the synthesis of benzimidazolones of general formula **A**, the pharmacomodulation realized for increased selectivity for the 5-HT₇ receptor, and studies on the efficacy of these ligands determined on the cAMP-mediated signalling pathway in a recombinant expression system.

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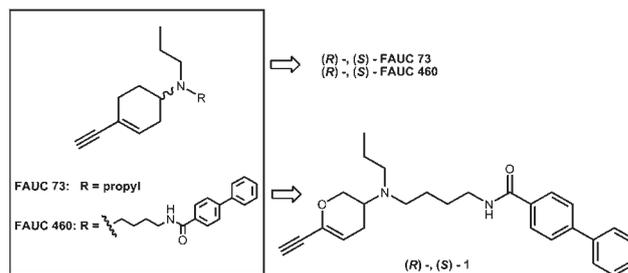
Enantiopure Cyclic Enyne Derivatives as Potent Dopamine D₃ Receptor Agonists

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The dopamine D₃ receptor subtype proved to be a promising therapeutic target for the treatment of neurological and psychiatric disorders, such as schizophrenia, Parkinson's disease, drug addiction and inheritable essential tremor.^[1] Due to these putative applications, the investigation of highly selective full and partial D₃ receptor agonists has been an active field of research over the last two decades.

Recently, we described non-aromatic conjugated π -systems as potent catechol bioisosters. The ethynylcyclohexene derivative FAUC 73 displayed excellent D₃ affinity together with partial agonist properties.^[2] Introduction of a lipophilic appendage leading to the biphenyl carboxamide FAUC 460 improved both D₃ affinity and selectivity while remaining the intrinsic activity.^[3]



Based on these results, we were intrigued by the question of whether these enyne-based ligands exhibit stereospecific interactions with the D₃ receptor binding pocket. Therefore, we established a methodology to obtain FAUC 73 and FAUC 460 in enantiopure

form. Additionally, employing an “ex-chiral pool” synthesis, we replaced the cyclohexenyl ring by a dihydropyran to improve the stereoselectivity of the ligand–receptor interactions. All synthesized enantiopure substances were examined for both binding and efficacy.

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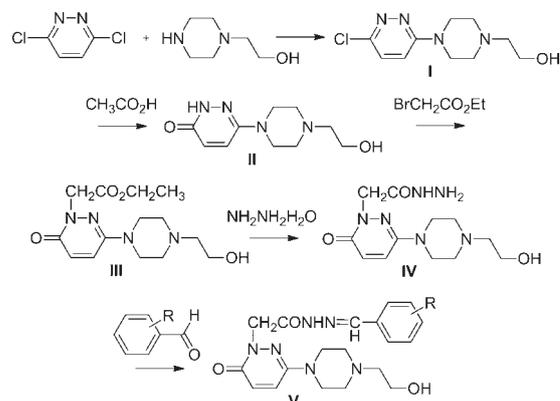
Synthesis and Antimicrobial Evaluation of 6-Substituted-3(2H)-pyridazinone-2-acetyl-2-substituted Benzalhydrazone Derivatives

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The pyridazinone nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs.^[1] Some of the present day drugs such as emorfazone (analgesic), pimobendan (positive inotropic, vasodilator), levosimendan (calcium sensitizer), imazodan (cardiotonic), zardaverin (cardiotonic) and medazonamide (antitussif) are the best examples for potent molecules possessing pyridazinone nucleus. Due to favorable presence of a pyridazinone moiety in known active structures, pyridazinone derivatives provoked a special interest in the search for new antibacterial agent.^[2,3] Also, it is well known that the hydrazone group plays an important for the antimicrobial activity a number of hydrazone derivatives have been claimed to possess interesting antibacterial and antifungal activities. Considering the above, we report synthesis of fifteen of 6-(4-hydroxyethylpiperazine)-3(2H)-pyridazinone-2-acetyl-3-(substituted/nonsubstituted)benzalhydrazone derivatives by the condensation of 3(2H)-pyridazinone-2-acetohydrazides with substituted benzaldehyde derivatives. The structures of these new pyridazinone derivatives were confirmed by their IR, ¹H NMR spectra and elementary analysis. Antimicrobial activities of the synthesized compounds were also investigated.



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P582

Carrier-Free Surface Crystallized Drug Eluting Stent

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Coronary stenting has revolutionized current perspective of coronary artery disease management. Intense work on stent development has successfully led to the introduction of drug-eluting stents (DES) in 2002. This work presents a carrier-free DES, based on crystalline drug coating (Figure 1). The work presents rapamycin as model drug, which is a macrolide and used to prevent organ rejection and also found to have significant antiproliferation properties. Rapamycin crystals onto stent gradually released the drug over a period of several weeks in buffer media. Rapamycin crystal coating displayed stability and biocompatibility. Additionally, the controllability of crystallization process enables the generation of a variety of morphologies, physical states and coating thickness. In vivo experiments did not raise any obvious safety concerns, no evidence for the presence of necrosis or any inflammatory reaction. This process was further implemented using different drugs and supersaturated systems.

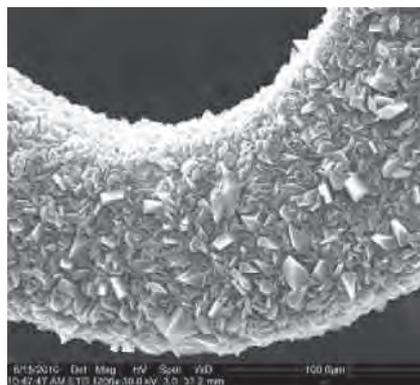


Figure 1. Rapamycin crystals onto metal stent.

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Design and Evaluation of Ebselen-Loaded Polymeric Micelles

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During the last few decades, it was found that synthetically available organoselenium compounds are active immunostimulants, inhibitors of enzymes, antioxidants, anti-inflammatory, antitumor, antiviral and antimicrobial agents.^[1–5] Although these compounds have a great potential as new perspective pharmaceuticals, their practical use is still limited due to low solubility in water. Thus, the purpose of our study was to develop an effective nano-carrying system for ebselen—an organoselenium compound with high biological activity. We designed and characterized ebselen-loaded polymeric micelles based on commercially available block copolymers of ethylene oxide and propylene oxide. Polymeric micelles were prepared by thin-film hydration method in several proportions in order to confirm optimal value of variables, i.e. polymers mass fraction and concentration, amount of ebselen, amount of water and level of hydration temperature and characterized by size, polydispersity and Zeta potential.

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P584

Mixed Polymeric Micelles as Carriers For 5H-Indolo-[2,3-b]quinolines

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Indoloquinoline alkaloids represent an important class of antimalarial, anti-inflammatory, antihyperglycemic, antibacterial and antiviral compounds.^[1] Moreover, it has been shown that 5H-indolo[2,3-b]quinolines—synthetic analogues of neocryptolepine, an alkaloid traditionally used in African folk medicine—are effective DNA intercalators and inhibitors of topoisomerase II, thus being promising anticancer agents.^[2,3] Although these compounds are highly active antiproliferative agents, their practical use is still limited due to their low solubility in water. So far, much effort has been put in to the development of more soluble derivatives^[4] or incorporating active substances into liposomes,^[5] however, there are no reports on polymeric micelles use. Thus, the purpose of our study was to develop an effective nanocarrying system based on commercially available block copolymers. We successfully designed and characterized mixed polymeric micelles that may be considered as nanocarriers for 2,5,9,11-tetramethyl-5H-indolo-[2,3-b]quinoline and 5,11-dimethyl-5H-indolo-[2,3-b]quinoline.

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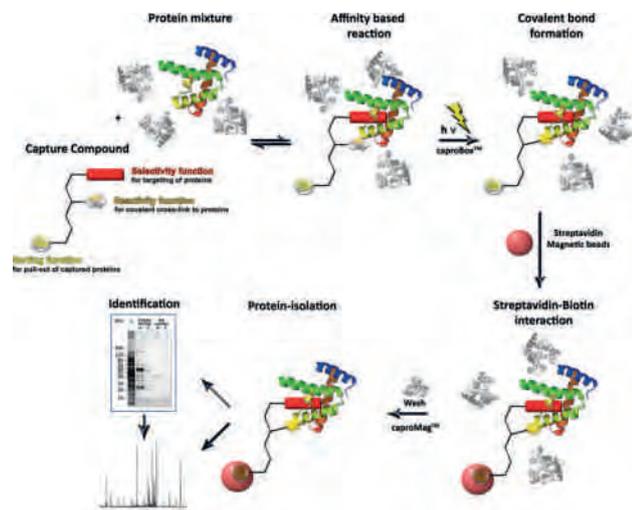
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P585

Capture Compound Mass Spectrometry (CCMS) Application in Thrombin and COMT Inhibitor Development

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For drug development, medicinal chemists are in great need to specify the protein target of drug candidates and to predict toxic side effects. As a unique cost and time saving technology, capture compound mass spectrometry (CCMS) addresses these challenges by the swift and unambiguous isolation and identification of proteins based on their specific interaction with small molecules (e.g. drug candidates) in any biological sample (cell lines and tissues) and in almost native environment.

The capture compound selectivity function (red) binds target proteins through reversible affinity interaction. A covalent bond between the photo-reactivity function (orange) and the protein(s) is generated through a photo-induced cross-linking reaction. The cross linked proteins can be isolated via the biotin sorting function (yellow) using streptavidin magnetic beads for Western blot or MS analysis. Offering an exceptional level of detection sensitivity, the CCMS process is highly reproducible and results can be obtained within 24 hours.

CCMS in Target Identification/Repurposing:^[1] Applying CCMS, thrombin as the known target of the particularly safe anticoagulant Dabigatran could be robustly isolated and identified with CCs derived from Dabigatran. Additionally, NQO2, HNMT, HYOU1, HNRPC, ADK, LRC59 and VP35 were identified as specific interactors from HepG2 cell lysate, which may be of relevance for the drugs full mode of action.

CCMS in Drug Safety Evaluation/Lead Optimization:^[2] In addition to its known target catechol-*O*-methyltransferase (COMT), numerous off-targets were identified from the soluble fraction of rat liver

applying Tolcapone-CCs. The major interactors occurred to be components of the respiratory chain (ATP5I, ATP5L, ATPB, ATPK, ATPO, ATPG, PRS8, PSME2, NDUA9, Cy1, QCR2, UCRI, UCRIL, DHSB, DHSB) and fatty acid β oxidation proteins (ECHA, ECHB, PRP8, SF381), revealing the molecular basis of Tolcapones hepatotoxicity. In current ImproMed™ studies, CCMS is directly used in profiling novel COMT inhibitors by means of activity and selectivity.

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P587

Synthesis, Characterization and Biological Evaluation of Pyrido[3,4-*c*][1,9]phenanthrolines as Novel Antitumor Agents

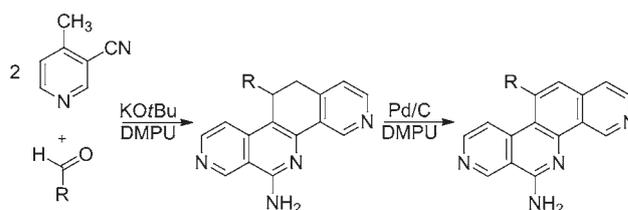
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With 7.6 million deaths in 2008 (WHO), cancer is one of the leading causes of death worldwide. This already high number is predicted to increase to 13.1 million deaths by 2030.^[1] Currently, many cancer diseases can neither be treated properly nor selectively by common drugs used in therapy. Moreover, cellular resistance to conventional cytostatics is a growing problem. Obviously there is a great demand for the identification and investigation of new active agents against cancer.^[2]

Topoisomerase I, an ubiquitous enzyme that is essential in mammals, is a clinically validated target for the development of new anticancer agents.^[3] In this regard, the tetracyclic aromatic benzo[*c*]phenanthridine alkaloids nitidine and fagaronine were found as model structures for the development of non-camptothecin DNA topoisomerase I inhibitors as they exhibit remarkable antitumor activity in vitro and in vivo.^[4] However, an essential drawback of the benzo[*c*]phenanthridines is their poor water solubility, limiting evaluation in cellular assays and the subsequent pharmaceutical use.

We developed a new class of previously undescribed cytotoxic heterocycles, the pyrido[3,4-*c*][1,9]phenanthrolines and 11,12-dihydro derivatives representing aza-analogous benzo[*c*]phenanthridines. The synthesis consists of an efficient one-step procedure leading to 6-amino-11,12-dihydro derivatives followed by an optimized dehydrogenation method to obtain the fully aromatic systems (see scheme).



Our compounds show promising results concerning reduced lipophilicity and increased solubility in aqueous media compared to the C-analogous benzo[c]phenanthridines. The cytotoxic potential of the substance class has been evaluated in a 60-tumor-cell-line screening at the US National Cancer Institute (NCI), in Maryland (USA), revealing remarkable growth inhibition for certain derivatives.^[5,6] These aspects highlight the pyrido[3,4-c][1,9]phenanthrolines as promising candidates to show efficacy in *in vivo* studies and/or other cancer model systems.

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P588

Translating the Favorable ADME Profile of a Lead Compound into Virtual Analogues in Restricted Physicochemical Space

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The efforts of lead optimization projects are directed towards analogues that have favorable ADME profiles and are devoid of safety concerns whilst retaining target activity. In this work, we present a novel computational platform to aid such projects by generating virtual analogue libraries in physicochemical space compatible with the desired biological characteristics.

The main idea behind our approach is that many considered properties are governed by basic physicochemical parameters, such as ionization, lipophilicity, and molecular size. We have devised simple yet accurate physicochemical models of intestinal absorption and passive permeation across the blood–brain barrier (BBB), and general physicochemical rules that hold even for protein–ligand interactions (P-gp, hERG inhibitor specificity). Changing parameter values may have distinct, even opposing effects on different ADME properties, and the impact of a particular parameter may depend on the allowed variation ranges of others. Using the cumulative output of available predictive models enables us to account for the multitude of possible effects and identify regions in physicochemical space that are most likely occupied by analogues with the desired combination of ADME properties. Advanced techniques are also applied to improve the selection of substituents fitting these regions, including custom Hammett equations for estimating mutual effects of the core molecule and the modified substituent on the analogue's pK_a .

The presented methods coupled with automatic analogue generation in accordance with imposed physicochemical restrictions, make our software platform a valuable tool to guide drug discovery projects towards the most promising candidates.

P589

In Vivo SPECT Imaging of a Newly Developed and Radiolabeled [¹²³I]-Iodopentamidine Prodrug for the Treatment of African Trypanosomiasis

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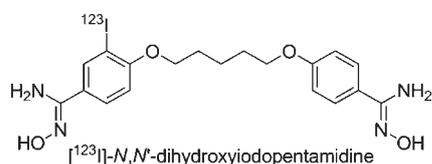
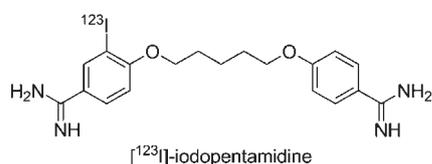
Background: Pentamidine is an effective antimicrobial agent and approved drug for the treatment of African trypanosomiasis (sleeping sickness). Due to the strong basic amidine moieties, pentamidine suffers from poor oral bioavailability and lacks CNS delivery limiting its applicability for *i.v.* or *i.m.* treatment of first-stage African trypanosomiasis. To improve oral and CNS bioavailabilities of pentamidine, many prodrug approaches have been pursued by our group to overcome this substantial problem.^[1–3] The aim of the work presented here was to perform *in vivo* imaging studies with a newly developed and radiolabeled pentamidine prodrug to examine its oral bioavailability and biodistribution.

Materials/Methods: First, iodinated reference substances were synthesized and characterized by NMR and LC/MS.^[4] Radiolabeling with [¹²³I] via isotopic exchange reaction was accomplished using either ammoniumsulfate in acetic acid for iodopentamidine or copper(I) chloride in DMSO for *N,N'*-dihydroxyiodopentamidine.^[4,5] Identification, separation, purification and quality control of the tracers were done by HPLC. In the imaging experiments, the distribution and bioavailability of intravenously and perorally administered tracers were compared using 12 male SD rats for each substance ($n=6$ *i.v.*, $n=6$ *p.o.*) over a period of 24 hours. After the last single-photon emission computed tomography (SPECT) images had been acquired, rats were perfused, disembowelled and γ -radiation levels of the organs were determined with a γ -counter. Analysis of reconstructed SPECT images was performed with PMOD software.

Results: The data showed that [¹²³I]-iodopentamidine was mainly renally eliminated, while [¹²³I]-*N,N'*-dihydroxyiodopentamidine was metabolized in the liver and underwent biliary elimination. Analysis of organ AUCs from processed SPECT images, as well as organ activity levels, suggested that the relative bioavailability of orally administered prodrug compared to orally administered [¹²³I]-iodopentamidine was significantly improved (e.g., 20.5% versus 0.7% (liver), 13.1% versus 2.2% (heart), 23.0% versus 6.1% (lung), 18.4% versus 2.1% (brain)). The specific activity levels of

intravenously injected [^{123}I]-iodopentamidine were set to 100%. Furthermore, a seven-times higher activity in the brain was observed after i.v. injection of [^{123}I]-*N,N'*-dihydroxyiodopentamidine compared to i.v. injection of [^{123}I]-iodopentamidine. In general, activity levels of all organs (except for kidneys and spleen) were significantly higher after prodrug than after [^{123}I]-iodopentamidine injection.

Conclusion: In conclusion, [^{123}I]-*N,N'*-dihydroxyiodopentamidine exhibited a highly improved oral bioavailability supporting the possibility for developing oral drug formulations that can be used to treat patients suffering from sleeping sickness. Notably, even the CNS bioavailability was apparently improved. However, additional efforts will need to be considered to further increase the drug concentration in the brain for the effective treatment of second-stage African trypanosomiasis.



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P590

^{18}F -LC40: A Highly Selective Radiotracer for the Imaging of Neurotensin Receptor 1 (NTS1)-Positive Tumors

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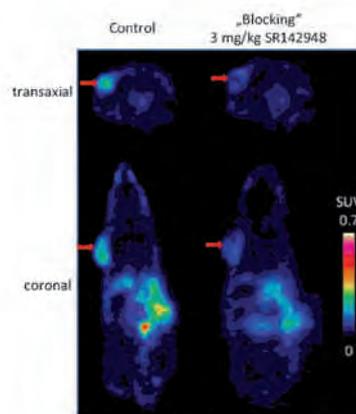
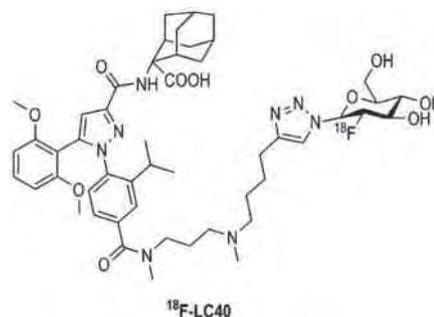
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Neurotensin (NT) is an endogenous, 13-amino-acid peptide that mediates a number of pharmacological effects involving dopamine transmission, analgesia, and hypothermia.^[1] In addition to these attributes, NT acts as a key player in multiple steps of cancer progression in numerous types of cancer cells.^[2] Those carcinogenic effects are presumably evoked by an abnormal expression of the neurotensin

receptor 1 (NTS1). Based on this overexpression, we aimed for a specific labeling of tumors by molecular imaging techniques such as positron emission tomography (PET).

We recently reported the design and synthesis of a radiolabeled derivative of the hexapeptide NT 8–13, the active fragment of the endogenous agonist neurotensin.^[3] As an extension of these investigations, we planned to construct a ^{18}F -labeled non-peptidic NTS1-selective ligand based on the potent NTS antagonist SR142948A.^[4] Herein, we report the novel PET tracer ^{18}F -LC40, which could be synthesized by taking advantage of our click-chemistry-based ligation of 2-deoxy-2- ^{18}F fluoroglucosyl azide (azido- ^{18}F FDG) to the alkyne-functionalized analogue of the lead structure. Receptor binding experiments using NTS1-expressing CHO cells indicated a K_i value of 0.5 nM for F-LC40 with 70-fold selectivity towards NTS2. The precursor for ^{18}F -labeling was obtained by a palladium-catalyzed aminocarbonylation^[5] of the respective bromoarene derivative with an alkynylamine using $\text{Mo}(\text{CO})_6$ as a carbon monoxide source. The copper-catalyzed azide-alkyne cycloaddition (CuAAC) using azido- ^{18}F FDG afforded the glycosyl ligand ^{18}F -LC40 in a radiochemical yield of 20% in a total synthesis time of 75 min.

The μPET studies using HT29 (human colon carcinoma cell line)-xenografted nude mice demonstrated specific binding of ^{18}F -LC40 in vivo. Furthermore, the tracer displayed a good tumor to blood ratio and excellent metabolic stability both in vitro and in vivo.



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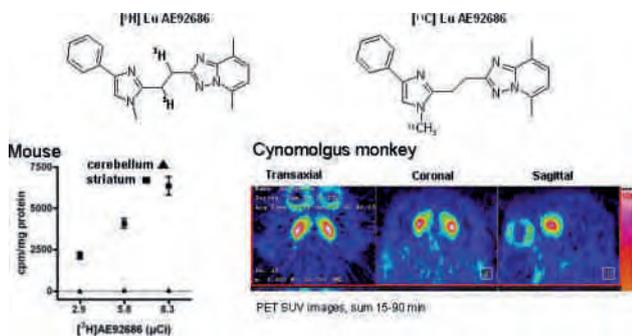
Discovery and Clinical Evaluation of [¹¹C]Lu AE92686 as a Radioligand for PET Imaging of Phosphodiesterase 10A in the Brain

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Phosphodiesterase 10A (PDE10A) has been proposed as a novel target for e.g. schizophrenia, and a PDE10A inhibitor could provide efficacy on positive, cognitive, and negative symptoms of schizophrenia.^[1] Numerous PDE10A inhibitors are currently undergoing clinical trials. In order to properly support target validation, for example, by allowing direct insight into the in vivo distribution of this enzyme and assist the clinical development of PDE10A inhibitors by providing evidence for a clinical candidate reaching and binding to the target, we have developed 5,8-dimethyl-2-[2-([¹¹C-1-methyl]-4-phenyl-1*H*-imidazol-2-yl)-ethyl]-[1,2,4]triazolo[1,5-*a*]pyridine ([¹¹C]Lu AE92686) as a novel PDE10A enzyme inhibitor PET radioligand.

Herein, we report the preclinical evaluation of Lu AE92686 in vitro, as well as in vivo in rodents as the corresponding tritiated radioligand, [³H]Lu AE92686, and in cynomolgus monkeys and humans as the ¹¹C-labelled radioligand, [¹¹C]Lu AE92686.



In short, Lu AE92686 displayed high in vitro affinity for the PDE10A enzyme ($IC_{50}=0.39$ nM) and selectivity >1000-fold over other PDE subtypes. Moreover, profiling of [¹¹C]Lu AE92686 in monkeys and humans suggests that it has great potential as a human PET radioligand for the evaluation of the occupancy of compounds targeting the PDE10A enzyme.

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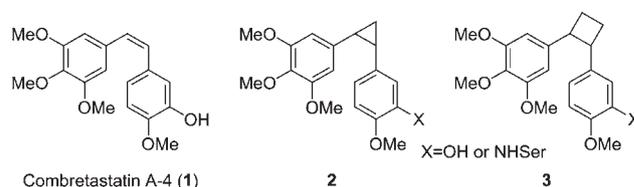
Novel Combretastatin A-4 Analogues: Synthesis and Biological Evaluation

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Combretastatin A-4 (**1**) was isolated from the African willow tree *Combretum caffrum* by the Pettit group in 1982.^[1] The natural product shows promising biological activity by binding to the colchicine binding site on tubulin, which leads to the inhibition of microtubule polymerization as well as the selective shutdown of the tumor blood flow. Due to cytotoxic effects against a wide range of human cancer cell lines, derivatives of CA-4 are already used in clinical studies.

To avoid the disadvantage of rather low in vivo efficacy resulting from the isomerization of the *cis*-stilbene derivative to the thermodynamically more stable *trans*-isomer, analogues containing carbocycles with different ring sizes (**2**, **3**) that prevent the system from undergoing *cis*–*trans* isomerization were prepared.^[2]



Derivatives containing three- and four-membered carbocycles with varying degrees of saturation replacing the double bond revealed extremely high levels of activity in the nanomolar range. Syntheses of these new derivatives of CA-4 as well as docking studies and results from biological activity studies against human cancer cells are discussed.

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Developing Fungal Chemosensitizers to Combat Fluconazole Resistance in *Candida albicans*

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The US National Institutes of Health (NIH) Molecular Libraries and Probe Production Centers Network (MLPCN) performed a high-throughput phenotypic screen of >300,000 compounds to identify chemosensitizers of fluconazole-resistant *Candida albicans* clinical isolates. Several chemical scaffolds were selected for further optimization, and subsequent structure–activity relationship investigations produced three structurally distinct small-molecule probes for interrogating resistance acquisition in *C. albicans*.

P594

Identification of a Small-Molecule Inhibitor of *Pseudomonas aeruginosa* PvdQ Acylase, an Enzyme Involved in the Synthesis of the Siderophore Pyoverdine

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The bacteria *Pseudomonas aeruginosa* produces a peptide siderophore known as pyoverdine, which has a great affinity for Fe³⁺ and is used to acquire this essential metal from the external environ-

ment. Several enzymes such as the PvdQ acylase are required for the biosynthesis of pyoverdine. Deletion of the PvdQ gene disrupts pyoverdine production and hinders *P. aeruginosa* proliferation. Bacteria defective in pyoverdine synthesis are not infectious, suggesting that a disruption in siderophore production through PvdQ inhibition could be exploited as a target for the development of novel antibiotic compounds. A small-molecule inhibitor of PvdQ acylase has been developed. The optimized compound inhibits PvdQ in vitro with an IC₅₀ value of 6 nM, has no apparent toxicity in mammalian HeLa cells up to a concentration of 100 μM, and inhibits growth and pyoverdine production in *P. aeruginosa* exposed to iron-limiting conditions with an IC₅₀ value <50 μM. A solved crystal structure of the inhibitor bound to PvdQ illustrates on-target mechanism of action. Small-molecule inhibition of PvdQ significantly reduces the intracellular uptake of iron inside the bacteria and is a useful tool for ongoing characterization of pyoverdine's role in *P. aeruginosa* biology.

P595

The Prismatic Puzzle of the Simple Neuraminic Acid Glycol Allylic Substitutions

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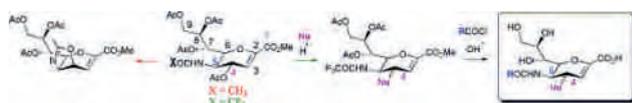
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The high chemical interest in derivatives of 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (DANA) is due to their wide biological and pharmacological applicability as transition-state intermediate-like inhibitors of viral, bacterial and mammalian sialidases.^[1] Indeed, on one hand, we dispose of clinically used drugs, such as Zanamivir, and many other compounds currently in clinical trials, all sharing DANA structure and possessing high antiviral or antibacterial activity; on the other hand, the discovery of a selective antagonist of human sialidases represents a very interesting biochemical and pharmacological goal. So, many protocols are developed to easily synthesized new DANA-substituted glycals, especially at the C-4 and C-5 positions, key sites for the enzyme–substrate interaction. However, despite these efforts, at present, no general method to rapidly substitute DANA glycals is available. Thus, the aim of this work is to study the acid-mediated allylic substitutions on DANA and on its *N*-perfluorinated congener (FANA) derivatives in order to set up an extensive and straight protocol to afford a high number of potentially effective sialidase inhibitors.

As proven in our last work,^[2] we can utilize the protected FANA as key tool to bypass oxazoline derivative, normally formed by acidic treatment of peracetylated DANA. In fact, the electron-withdrawing effect of fluorine atoms deactivates the C-5 *N*-carbonyl, preventing the intramolecular acetamido nucleophilic attack, and promotes the external nucleophile substitution at the allylic carbocation at C-4. As expected, we set up an innovative and widely applicable protocol to insert many nucleophiles (alcohols, thiols, halogens, sulfonamides) at the C-4 position. In addition, these molecules, bearing a labile group at C-5, represent the appropriate precursors of new classes of

C-4 and C-5-modified glycals, easily derivable from a mild hydrolysis of esteric and amidic functions followed by a selective acylation of amino group at C-5.

However, behind its simple appearance, this work hides many variables we have to consider to comprehend the reaction mechanism and to eventually improve the regio- and stereo-selectivity. First of all, we investigate the association with the Ferrier reaction,^[3] followed by the possible 2,4-nucleophile shift, to discover any internal rearrangement. Then, we examine the glycerol chain protecting group influence related to the reaction efficiency and to the regio-/stereo-isomeric ratio of the products. Finally, we evaluate the protected DANA and oxazoline derivatives as potential reaction precursors, monitoring their behavior when subjected to the same protocol.



Every piece of this intricate puzzle is arranged into a rational explanation, and the final successful results are reported.

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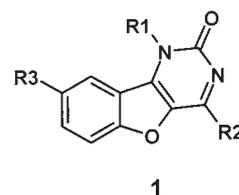
P596

Identification and Optimization of a Novel HIV Nucleotide-Competing RT Inhibitor Series

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Several groups have recently reported on the identification of nucleotide-competiting reverse transcriptase inhibitors (NcRTIs), a new class of RT inhibitors. NcRTIs reversibly inhibit binding of the incoming nucleotide to the RT active site but do not act as chain terminators, unlike the nucleos(t)ide reverse transcriptase inhibitor (NRTI) class. In this presentation, we will highlight our research in the identification and optimization of a novel series of NcRTIs, resulting in the discovery of inhibitors with excellent antiviral activity and drug-like properties.



Screening of our corporate database led to the identification of a benzo[4,5]-furo[3,2,d]-pyrimidin-2-one hit (exemplified by **1**) with moderate biochemical activity. SAR studies around this hit provided analogues with significantly improved biochemical and antiviral activity. However, these compounds had poor metabolic stability, very low permeability, high efflux, and low bioavailability in rats. Extensive SAR studies revealed that some of these issues could be resolved by reducing the pK_a of the most basic amine in the lead inhibitors. An equally important observation during the course of our studies was the need to shield polar functionality in order to optimize the ADME properties of the inhibitors. This presentation will provide an overview of the key findings from our studies that ultimately led to the discovery of novel NcRTI inhibitors with excellent antiviral potency, and good pharmacokinetic and drug-like properties.

P597

Discovery, Synthesis and Mechanism of Action of Bicyclic Anti-influenza Agents

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History warns humans that influenza pandemics, such as those in 1918 (H1N1), 1968 (H3N2), 2005 (H5N1), and 2009 (H1N1), could have a serious social and economic impact. Under the current public health threats from seasonal influenza pandemics or epidemics and outbreaks of resistant-influenza strains, novel antiviral agents with new mechanism of actions are urgently needed. In our ongoing search for antiviral agents, we have performed a high-throughput screening (HTS) with a chemical library from the Korea Chemical Bank to discover hit compounds active against influenza viruses. Through a series of HTS and evaluation of activity and toxicity profiles of the compounds screened, a bicyclic small molecule was discovered and confirmed as a hit. To optimize the hit compound, various analogues related to this hit compound were prepared and their antiviral activities against Type A (H1N1 and H3N2) and Type B viruses were evaluated. We will present the synthesis of the bicyclic compounds, the structure–activity relationships, and preliminary results on the mechanism of action of the compounds prepared.

P598

Design and Synthesis of Multifunctional Ligands Endowed with Antiproliferative Activity

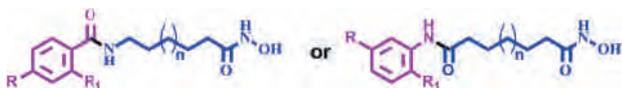
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Over the past years, we have been engaged in a project aimed at identifying novel biologically active small molecules, which can be either antitumor leads candidates or valuable chemical tools to study molecular pathways in cancer cell. As a part of this project, in view of the multifactorial mechanistic nature of cancer, we planned to develop some hybrid compounds using the multitarget-directed drug design strategy.

According to this approach, we synthesized new chimeric molecules by linking together suberoylanilide hydroxamic acid analogues, targeting histone deacetylases, and variously substituted stilbene, biphenyl or terphenyl derivatives able to block the cell cycle, and induce apoptosis and cell differentiation.^[1,2] The distinct synthons were separately synthesized following different protocols previously described by us,^[1,2] and linked via an amide bond taking advantage of appropriate coupling conditions. The new compounds are currently under biological study for their ability to interfere with cell cycle progression and to inhibit HDACs.



Acknowledgements: This work was supported by a PRIN2009 Project Grant from MiUR, Italy.

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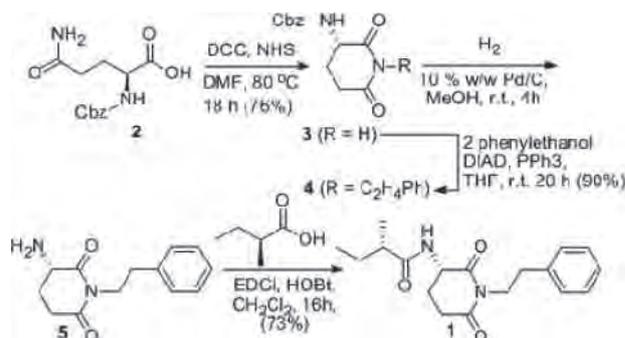
Target and Diversity Oriented Approach towards (–)-Julocrotine and Analogues

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Julocrotine (**1**) is a natural glutarimide alkaloid isolated from several plants of the genus *Croton*, including *Croton cuneatus* Klotzsch.^[1] Recently, it could be identified as a growth inhibitor of promastigote and amastigote forms of the protozoan *Leishmania amazonensis* (L.) with

no cytotoxicity against the host cell.^[2] After analyzing the structure of (–)-julocrotine, we set out to synthesize it in only three steps from commercially available L-Cbz-glutamine **2** (see scheme). Intermediate **5** was also utilized as the amino building block in Ugi four-component reactions for the preparation of julocrotine analogues.



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P600

5-Hydroxymethylcytosine and Its Oxidized Congeners—Modified Bisulfite Sequencing Methods and Mechanistic Insights

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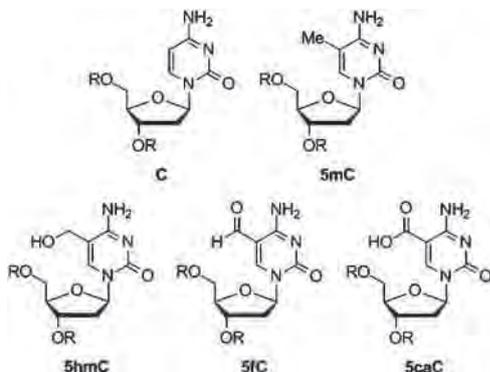
5-Methylcytosine (5mC) plays an important role as an epigenetic mark. Recently, it was shown that the ten-eleven-translocation (TET) enzymes convert 5mC to 5-hydroxymethylcytosine (5hmC) and also generate its oxidized congeners 5-formylcytosine (5fC) and 5-carboxycytidine (5caC), thus, offering efficient demethylation pathways.

After the recent development of oxidative- and TET-assisted bisulfite sequencing protocols, the genome-wide mapping of 5hmC at single-base resolution becomes feasible.^[1,2] This will provide a complete and more detailed overview of the epigenetic profile of mammalian cells and result in a more detailed understanding of the role of 5hmC and 5mC.

In order to determine whether 5fC and 5caC play a significant role either as intermediates in demethylation processes or as “new” epigenetic marks, a base-pair resolution sequencing method is needed.

The mechanistic rationale for the bisulfite-mediated deamination and decarboxylation reactions still remains sketchy. A reliable mechanistic explanation of these reactions might not only elucidate this “black box” procedure, but also give a hint to whether a second type of demethylation mechanism that does not rely on base excision

repair pathways is available. Here, we present our efforts towards this end, as well as a possible method to identify these oxidized 5hmC congeners.



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P601

Rapid Technique for New Scaffold Generation

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Scaffold hopping remains a central task in medicinal chemistry for generating and protecting intellectual property. We present a technique for rapidly generating reasonable yet novel scaffold replacements. Our technique uses the molecular interaction fields^[1] of the parent molecule and assesses replacements in the context in which they will be synthesized. This enables the differing steric and electronic effects of potential new scaffolds to be used. An added bonus of our method is that replacements for terminal substituents can be considered alongside more central moieties enabling its use in growing fragments and lead optimization as well as lead generation. The method is embodied in the software product “sparkV10” and will be presented together with a number of case studies. Direct comparison with the recently published NEAT method from Pfizer^[2] that utilizes quantum mechanical calculations will show that similar or better results can be obtained in a few minutes on a desktop PC. Limitations and future optimizations will be discussed. The use of our method for growing or linking fragments in fragment-based drug discovery will also be presented with respect to the preparation of selective ligands targeting a selective, slow off-rate kinase inhibitor for COPD. Advantages and disadvantages of our ligand-based method over structure-based methods will also be presented.

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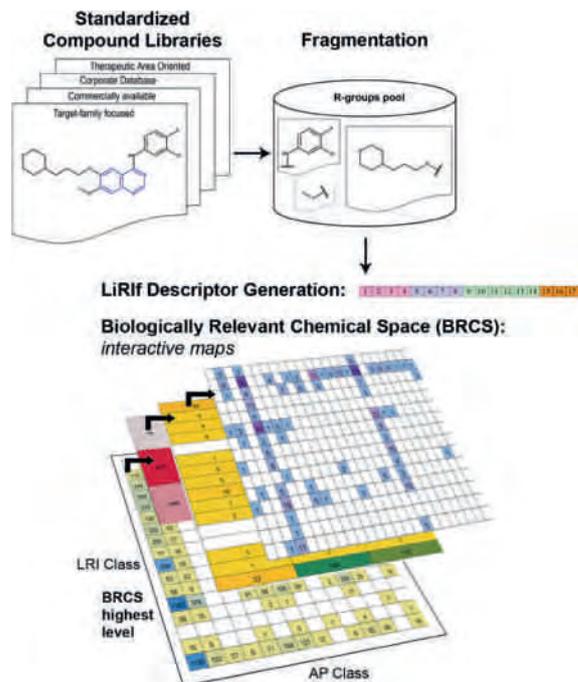
P602

Intuitive Med Chem Guidance Tool to Map Biologically Relevant Chemical Space

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Based on a recently reported descriptor (LiRif),^[1] we show a novel method for fast and intuitive interpretation of structure–activity relationships (SARs), patent analysis, library enrichment, and reagent selection for hit exploration. We analyze in detail the phthalazinamines series explored by different competitors to show these four different capabilities of this visualization tool.^[2]



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P603

Synthesis of 2-Propynylurea Derivatives as Anticancer Agents

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Novel 2-propynylureas were firstly designed in our lab as an in-house library for the preparation of 1,4-disubstituted-1*H*-1,2,3-triazoles by copper-catalyzed azide alkyne cycloaddition (CuAAC). These compounds were docked with vascular endothelial growth factor receptor-2 (VEGFR-2) template, and 11 hit compounds were identified from in silico screening. The identified 2-propynylureas were synthesized and initially screened for antiproliferative activities against human breast cancer cells (MCF-7) and human cervical cancer cells (HELA). Five compounds inhibited the growth of both cancer cell lines in a dose-dependent manner. 1-((3-Chloromethyl) phenyl)-3-(2-propynyl)urea (**1**) was the most active with IC₅₀ values of 1.55 μM and 1.48 μM against MCF-7 and HELA, respectively. This compound was evaluated for its antiproliferative effect against three more cancer cell lines including human hepatoma cells (HepG2), human small-cell lung carcinoma (NCI-H187) and human non-small-cell lung carcinoma (H460). The IC₅₀ values of compound **1** against HepG2, NCI-H187 and H460 were found to be 76.56 μM, 0.37 μM and 78.34 μM, respectively. Kinase inhibition of compound **1** against VEGFR-2, epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor-β (PDGFR-β) was evaluated, and it predominantly inhibited the phosphorylation of EGFR. The cytotoxicity of **1** against the EGFR-overexpressing cell line A431 (IC₅₀=36 nM) was comparable to that of erlotinib. The binding mode of compound **1** from docking simulation in the binding site of EGFR kinase revealed that the urea motif formed hydrogen bonding with Lys745, Thr854 and Asp855 in hydrophobic pocket of EGFR. Compound **1** is considered as a potential lead for anticancer drug development.

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P604

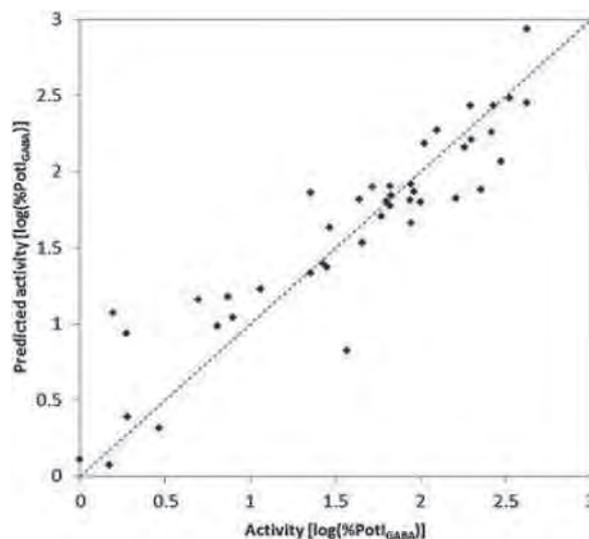
Valerenic Acid as a Model Compound for New GABA_A Receptor Ligands

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Root extracts of *Valeriana officinalis* are long known in traditional medicine for inducing sleep and the treatment of panic disorders. Valerenic acid, a chiral bicyclic sesquiterpene, was found to act as a subtype-selective positive allosteric modulator of GABA_A receptors bearing β2 or β3 subunits.^[1] Therefore, it is mainly responsible for the desirable activity of valerian.

The synthesis of valerenic acid requires exact control of three stereocenters and the use of the cost-intensive Crabtree's catalyst. Thus, a simplified scaffold for the design of druggable compounds should be identified. To this end, the activity-related structural motifs need to be discovered by both chemical synthesis and computational methods.



We present a comprehensive 3D QSAR model based on a congeneric dataset of 42 valerenic acid derivatives. The atom-based 3D QSAR function implemented in Phase/Schrödinger was used. All investigated compounds were aligned according to the best fit to the diaxial arrangement of both on-ring substituents, as found in the X-ray structure of valerenic acid. The original dataset was randomly divided into 80% training and 20% test set multiple times, and the best performing model was chosen to make predictions within a small dataset of related structures. New derivatives were predicted with an *R*² value of 0.87 and a *Q*² value of 0.73. The quality of the model was tested via biological testing of synthesized compounds.

Acknowledgement: This work is kindly financed by the FWF project no. W1232, Molecular Drug Targets.

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P605

The Mammalian Molybdoenzyme mARC—Localized in Peroxisomes?

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Hypothesis: The mitochondrial amidoxime reducing component (mARC) is a molybdenum-cofactor containing enzyme.^[1] mARC is part of a mitochondrial enzyme system together with cytochrome b5 and its reductase, and this enzyme system is located on the outer mitochondrial membrane.^[2,3] Mammalian genomes encode for two mARC enzymes (mARC1/mARC2), and both mARC enzymes are able to reduce *N*-hydroxylated compounds together with cytochrome b5 and b5 reductase.^[4] Although the contribution of the *N*-reductive enzyme system in drug metabolism pathways is well accepted, the physiological function of mARC is not fully understood. Interestingly, proteomic characterization of peroxisomes and immunocytochemically visualized Myc-tagged protein versions suggested a peroxisomal localisation of mARC2 in mammalian cells.^[5,6]

Methods: Subcellular fractions from mammalian livers were analysed by Western blot. Furthermore the *N*-reductive activity of these fractions was determined using the marker substrate (benzamidoxime) of the *N*-reductive enzyme system.

Results: By this approach, additional evidence of a peroxisomal localisation of mARC2 was provided. Furthermore it is the first time that mARC was detected in a subcellular fraction without the electron-transfer proteins cytochrome b5 and its reductase. In consequence, *N*-reductive activity was not enriched in the peroxisomal fraction. In contrast, both mARC proteins were enriched in mitochondria, but only mARC2 was enriched in the outer mitochondrial membrane.

Conclusion: In peroxisomes, mARC seems to be involved in so far unknown redox reactions either as a stand-alone protein or with other electron-transport proteins different from cytochrome b5 and its reductase.

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P606

Reversal of Tumoral Immune Resistance by Inhibition of Tryptophan 2,3-Dioxygenase (TDO): Design, Synthesis and Preclinical Evaluation of a Novel TDO Inhibitor

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Tryptophan catabolism mediated by indoleamine 2,3-dioxygenase (IDO1) is an important mechanism of peripheral immune tolerance contributing to tumoral immune resistance,^[1] and IDO1 inhibition is an active area of drug development.^[2] Recently, the team of Benoit Van den Eynde (LICR, UCL, Belgium) has shown that tryptophan 2,3-dioxygenase (TDO), an unrelated hepatic enzyme also catalyzing the first step of tryptophan degradation, is also expressed in many tumors and that this expression prevents tumor rejection by locally depleting tryptophan.^[3] In this communication, a detailed structure–activity study of a series of 3-(2-(pyridyl)ethenyl)indoles as TDO inhibitors will be presented. This study led to the identification of a potent and orally available TDO inhibitor (LM10) that, upon systemic treatment, restored the ability of mice to reject TDO-expressing tumors. Our results thus describe a new mechanism of tumoral immune resistance based on TDO expression and establish proof-of-concept for the use of TDO inhibitors in cancer therapy.^[4,5]

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P607

Discovery of Novel Binders of Bromodomain BRD4

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Introduction: Bromodomain-containing proteins are of biological interest as substantial components of transcription factor complexes and determinants of epigenetic control. They specifically recognize acetylated Lysines on histone tails, thus influencing the expression of genes. Not surprisingly, the therapeutic relevance of these protein–protein interactions has been shown for several disorders.^[1] For example, the BET bromodomain family member BRD4 has been proposed as a promising pharmacological target in cancer.^[2] To date, only a few binders and active compounds have been described for this protein.^[2,3] Several crystallographic structures are available, allowing for the rational structure-based discovery of novel inhibitors of BRD4. Here, we present the results of a virtual screening experiment followed by in vitro validation performed on this protein.

Methods: A collection of more than 9 million unique drug-like small molecules was tested virtually in the binding pocket of the first bromodomain of BRD4 using Glide 5.6 (Schrödinger Inc.). We expressed the first bromodomain of human BRD4 in an *E. coli* strain and performed ITC measurements to assess the in vitro affinity of a set of purchased compounds selected from the in silico experiment.

Results: We have identified small molecules with novel chemical scaffolds that exhibit affinity for BRD4. These compounds have been co-crystallized with the protein, thus providing us with valuable and detailed information on binding. The molecules will be further analyzed and subjected to an optimization process, in order to identify chemical substitutions that would increase their binding affinity for the target. The ability of the identified compounds to disrupt the interaction between BRD4 and peptide models of acetylated histone H3 will be studied by DSC and SPR measurements.

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An Integrative In Silico Guided Platform for the Rational Design of Protein–Protein Interaction Modulators

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The modulation of protein–protein interactions (PPIs) with small chemical probes is a relatively new area of research with promising therapeutic potential. The rational design of small-molecule modulators of PPIs is however challenging because: i) PPIs can occur through multiple interaction sites (hot spots), and ii) PPI interfaces are flexible.

We have developed an integrative platform for the rational design of chemical probes that target the interaction between the von Hippel–Lindau (pVHL)–Cullin Ring-type E3 ubiquitin ligase (CRL) and Hypoxia inducible factor 1 alpha subunit (Hif-1 α).^[1] This PPI is a therapeutic target for the treatment of chronic anemia (associated with kidney disease and cancer) and acute ischemia. By combining structural, biophysical and chemical techniques we have designed: i) molecules that can disrupt the pVHL:Hif-1 α interaction,^[2] and ii) PROteolysis TARgeting Chimeric (PROTAC) molecules that may enable the degradation of proteins of interest by the proteasome.

Here, I will describe how we introduce complementary ligand-based and protein-based computational methods to further improve the binding affinities and drug-like properties of our chemical probes and exploit them as anchors of novel pVHL-targeting PROTAC molecules.

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Generative Topographic Mapping: A Universal Approach for Data Processing in Chemoinformatics

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This presentation concerns generative topographic maps (GTM),^[1] which is a universal tool to visualize chemical space, to develop classification models, to estimate the applicability domain of models and to compare databases of chemical compounds.^[2] The model calculations performed on the DUD^[3] and human ether-a-go-go related gene (HERG) channel inhibitors datasets using different types of descriptors to illustrate the utility of GTM.

As a tool for visualization, GTM overcomes most of drawbacks of such popular approaches as principal component analysis (PCA), sammon mapping (SM) and self-organizing maps (SOM). The probabilistic character of the GTM approach appears to offer additional advantages, which directly stem from the rigorous probabilistic character of the GTM approach. Thus, GTM can also be used to assess an overlap between the datasets, to develop classification models if information about activities of compounds is available, and to assess the applicability domain of models.

In this study, we consider some aspects of GTM models for data visualization, the chemical interpretation of the maps and application of GTM as a tool for structure–property modeling, applicability domain estimation and data distribution comparison. Our ultimate goal is to present GTM as a unique universal approach for data processing in chemoinformatics.

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The Multifunctional Tryptoline and Tryptamine Triazole Derivatives that Enhanced the Neurite Outgrowth of Cultured P19-Derived Neurons

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Alzheimer's disease (AD) is a common neurodegenerative disorder for which one of the hallmarks is the deposition of aggregated β -amyloid peptides ($A\beta_{40,42}$) as plaques in brain. Oligomers of these peptides can react with biological metal in brain to generate free radicals resulting in neuronal cell death. We have previously reported tryptoline and tryptamine triazole derivatives (**6h**, **12c** and **12h**) as lead compounds acting on multiple targets, namely β -secretase (BACE1), β -amyloid peptides ($A\beta$), metal chelation and antioxidant. The multifunctional lead compounds inhibited BACE1, the key enzyme to generate β -amyloid peptides, and also interacted with $A\beta$ and prevented the amyloid self-aggregation to form amyloid oligomers and plaques. In addition, metal chelation and antioxidant properties helped in reducing radical formation and scavenged the generated radicals. The multifunctional activities of compound **6h** included anti-amyloid aggregation and antioxidant effects while those of compound **12c** were β -secretase inhibitory action, anti-amyloid aggregation and metal chelating. Compound **12h** acted as $A\beta$ aggregation blocker, chelator and antioxidant. As neurite dystrophy has been found in AD brain, and this significant loss of connectivity of neuron relates to cognitive decline, the multifunctional lead compounds (**6h**, **12c** and **12h**) were evaluated for neuritogenic activity using P19-derived neurons. The morphology of P19-derived neurons was observed and the length and number of neurites was measured comparing to geldanamycin, a positive control. At the noncytotoxic concentration of 1 nM, lead compounds **6h**, **12c** and **12h** showed significant increase in neurite length and neurite number. The results suggested that the multifunctional lead compounds not only acted as neuroprotectants against neurotoxicity from $A\beta$ on neuronal cells but also enhanced the survival and neurite outgrowth of P19-derived neurons.

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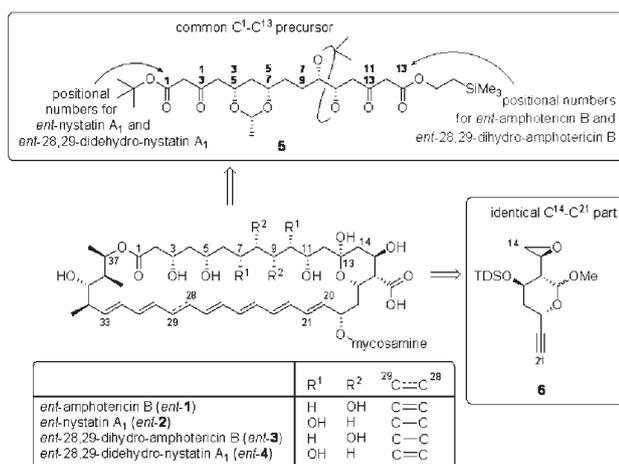
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Towards the Syntheses of the Unnatural Enantiomers of the Polyol–Polyene Antibiotics Amphotericin B, Nystatin A₁, Amphotericin A, and 28,29-Didehydro-nystatin A₁ by Common Precursors

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The polyol–polyene antibiotics amphotericin B (**1**) and nystatin A₁ (**2**), isolated from *Streptomyces nodosus*^[1] and *Streptomyces noursei*,^[2] respectively, are used as highly potent antimycotics for the treatment of fungal infections.^[3]



Currently we are working on the total syntheses of the unnatural enantiomers *ent-1* and *ent-2* of amphotericin B and nystatin A₁. Because of the similarity of the target molecules, their polyol parts (C¹–C¹³) shall be synthesized from a common precursor and their polyene parts (C²²–C³³) likewise. Conveniently, the “Western” (C³³–C³⁷) and “Eastern” moieties (C¹⁴–C²¹) are even pairwise identical. This approach should make it possible—by crosswise permutation of the polyol and polyene parts—to synthesize both *ent*-amphotericin A (*ent*-28,29-dihydroamphotericin B; *ent-3*) and *ent*-28,29-didehydro-nystatin A₁ (*ent-4*). The synthesis of the identical “eastern” part **6** was realized in 18 steps, containing a ring-closing metathesis to build up the six-membered ring. As a promising unified precursor for the polyol parts of *ent-1* and *ent-2*, we identified the bis(β-ketoester) **5**. It was synthesized in 20 steps in

the longest linear sequence. One key step of the synthetic approach is an asymmetric bis(dihydroxylation) of a diene, which results in a particularly enantioselective installment of four stereogenic centers in one operation. After asymmetric hydrogenation of both β-ketoester moieties within the advanced precursor **5**, we plan, as a second key step, to differentiate the β-ketocarboxyl moieties by using their orthogonal protecting groups.

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New Model and Software for the Prediction of Blood–Brain Barrier Permeability

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The ability to penetrate the blood–brain barrier is among the most important ADME parameters of drugs that control their bioavailability and play an important role in determining their activity. For CNS-targeted drugs, the permeability should be enhanced while for other drugs it should be minimized to avoid possible side effects. Substantial work has been devoted to the modelling and prediction of this property. However, the applicability and usefulness of available models is often diminished due to limited data sets, inaccurate data, and/or insufficiently validated modelling approaches.

We have attempted to build generally applicable predictive models for the blood–brain barrier permeability (LogBB) of diverse drugs and drug-like compounds. The fragmental descriptors of up to six atoms were used in the conjunction with back-propagation neural networks (BPNN) in the framework of NASAWIN software.^[1] The descriptor subset for BPNN modelling was preselected using fast stepwise multiple linear regression (FSMLR). The model predictivity was assessed by 5x4-fold double cross-validation procedure.

We have compiled, to our knowledge, the most complete data set based on the open quantitative LogBB data (significantly extended over the largest previously published sets). The values were verified, and errors were corrected against the original publications. On the other hand, inorganic and small organic molecules irrelevant to medicinal chemistry were excluded. The final dataset contained 510 diverse organic compounds. The optimal model has Q² value (double cross-validation) of 0.79 and RMSE of 0.34. The model is implemented in convenient predictor software.

For an additional check, we used the recently published dataset of 2053 compounds^[2] containing estimated qualitative values (BBB+/BBB-). The predicted LogBB values were converted to the qualitative scale using the LogBB cut-off value of -1. This procedure gives total accuracy of 0.80, sensitivity of 0.91, specificity of 0.42, and precision of 0.84. In other words, more than 80% of BBB+ compounds in this independent validation set were identified correctly.

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Elucidating the Role of the mARC-Containing Enzyme System in Human Cells by the Use of RNAi Experiments

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Mitochondrial amidoxime-reducing component 1 and 2 (mARC1 and mARC2) are newly discovered mammalian molybdenum-containing proteins. Along with the electron-transport proteins cytochrome b5 and NADH cytochrome b5 reductase, mARC is part of the N-reductive enzyme system that was already shown to be responsible for the reductive activation of several *N*-hydroxylated prodrugs.^[1–4] Therefore mARC apparently plays a major role in drug metabolism but its physiological relevance is not known yet.

By transfection of siRNA into HEK-293 cells, genes of the components of the N-reductive enzyme systems were silenced to elucidate their roles in vivo. With the knock-down of the isoforms of cytochrome b5, we were able to show that only the mitochondrial and not the microsomal isoform is the physiological partner of mARC in vivo, although it is possible to reconstitute the N-reductive system with both isoforms in vitro. mARC knock-down experiments were carried out to clarify if mARC1, mARC2 or both homologues are involved in N-reduction in HEK cells.

A possible physiological function of the mARC-containing system could be the detoxification of mutagenic base analogues as it was already shown that the reconstituted N-reductive system is able to reduce mutagenic *N*-hydroxylated base analogues in vitro (paper in preparation).^[5] With RNAi as a tool, we tested if the mARC-containing system could exhibit a significant role as a detoxification system of these base analogues in vivo.

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Distribution of mARC in Different Porcine Tissues

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Mitochondrial amidoxime-reducing component 1 and 2 (mARC1 and mARC2) are newly discovered mammalian molybdenum-containing proteins.^[2] These proteins represent a novel group of molybdenum proteins in eukaryotes as they are the catalytic part of a three-component enzyme system together with the electron-transport proteins cytochrome b₅ and its reductase. In mammals, this N-reductive enzyme system is located in the outer mitochondrial membrane, and it is well accepted that it is involved in N-reductive drug and xenobiotic metabolism.^[1–4] Although the major portion of drug-metabolizing enzymes is located in the liver, we detected high extrahepatic mARC expression levels, especially in kidney and thyroid, which correlate with their N-reductive activity. A first hint on the physiological function of mARC is the detoxification of *N*-hydroxylated base analogues.

While the reductase activity dependent on mARC is only located in mitochondria, there are indications that mARC2 is found in peroxisomes also.^[5,6] So further functions of mARC are possible. We analysed different porcine tissues to verify if expression of mARC1 and mARC2 correlates in all investigated tissues with the N-reductive activity or if one or both mARC proteins must have another function in a specific tissue. Therefore, we analysed porcine tissue homogenates by the use of immunoblotting with specific antibodies raised against mARC1 and mARC2. Furthermore, we determined N-reductive activity with help of the model substrate benzamidoxime.

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Design, Synthesis and Biological Evaluation of Novel Antitubulin Agents

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Antitubulin agents are among the most important anticancer drugs and some of them are in clinical use. Over the last decades, novel anti-mitotic agents with a dual mechanism of action have been discovered. In addition to blocking tubulin polymerization, these compounds (e.g., combretastatin A-4, CA-4, and the chalcone derivative MDL-27048) are also able to damage selectively the tumor vasculature, interrupting the blood flow in tumors, starving the cancer cells. CA-4 and chalcone MDL-27048 are potent antitubulin compounds able to act as vascular disrupting agents at low concentration. Because of their pharmacokinetic problems (low solubility and metabolic instability), there are many attempts to modify these two parent molecules in order to impart them with better pharmacokinetic properties.

Our research was focused on the discovery of novel potential antitubulin agents starting from CA-4 and the chalcone analogues MDL-27048 as lead compounds. Three strategies have been applied: 1) the use of virtual screening for the identification of novel antitubulin agents by using a seven-point pharmacophoric model;^[1] 2) the replacement of the double bond of CA-4 with a furan, thiophene and cyclopentene ring exploring the concept of the regioselective coupling of polyhaloheteroaromatic compounds;^[2] 3) the replacement of the double bond of the chalcone derivative MDL-27048 with a triazole by using the concept of click chemistry.^[3]

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H₁/H₄ Dual-Action Antagonists: A New Generation of Antihistamines

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Antihistamines are histamine H₁ receptor (H₁R) antagonists currently used to treat a number of disorders. They typically block the allergic and inflammatory effects that histamine causes after it has been released from the mast cells and other sites of storage. However, the histamine receptor family is larger than just the H₁R and consists of four distinct subtypes (H₁R–H₄R). Recently, evidence is accumulating in the literature that supports the concept of combination therapies consisting of a H₁R antagonist and a H₄R antagonist in a single preparation to treat histamine-induced pruritis.^[1] A recent publication also described a H₁/H₃ single-ligand dual-action antagonist acting on two histamine subtypes for the treatment of allergic rhinitis.^[2] Therefore, we became interested in exploring whether novel H₁/H₄ dual-action antagonists could be used for the treatment of histamine-induced itch. We started our lead discovery program by evaluating the H₁/H₄ affinity of a library of compounds designed to bind to both receptor subtypes. We found a series of compounds based on different scaffolds that combined good affinity for both the H₁ and H₄ histamine receptors. One of these compounds (VUF11838) was evaluated in a histamine-induced itch model and demonstrated a significant additive effect compared to a selective H₄ antagonist.

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P617**Pharmacophores and Their Efficient Use for Ligand Profiling**Thierry Langer,^[a] Sharon D. Bryant,^[b] Gerhard Wolber^[b]

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In silico or virtual screening has gained considerable impact for the efficient discovery of novel bioactive compounds in modern pharmaceutical research. The concept of chemical feature-based pharmacophore models has been established as state-of-the-art technique for characterizing the interactions between a macromolecule and a ligand. The results of numerous case studies have been published, clearly indicating the merits of this approach for efficient hit discovery.^[1]

While in ligand-based drug design, feature-based pharmacophore creation from a set of bioactive molecules is a frequently chosen approach; structure-based pharmacophores are still lacking the reputation to be an alternative or at least a supplement to docking techniques. Nevertheless, screening using 3D pharmacophores as filters has the advantage of being faster than docking. Additionally, it transparently provides the user with relevant information that is used by the screening algorithms to characterize the ligand–macromolecule interaction.

At Inte:Ligand GmbH, LigandScout^[2] has been developed as a rapid and efficient tool for automatic interpretation of ligand–protein interactions and subsequent transformation of this information into 3D chemical feature-based pharmacophore models. As an extension of this approach, we have introduced parallel pharmacophore-based screening as an innovative in silico method to predict the potential biological activities of compounds by screening them with a multitude of pharmacophore models.^[3] Using LigandScout, the entire Protein Data Bank (PDB) has been processed, and a pharmacophore database of structure-based pharmacophore models for all targets of potential interest for drug development has been generated, in addition to ligand-based models for targets that lack information about their 3D structure.

We present an overview of this technology together with the results of an application example employing a set of antiviral compounds that were submitted to in silico activity profiling using a subset of the Inte:Ligand pharmacophore database. The results of the screening experiments show a clear trend towards correct prediction of activity profiles. In addition, using our approach, one is able to obtain information about binding of the ligands under investigation also to 'anti'-targets, such as enzymes of the cytochrome P450 family,^[4] or to the hERG channel. Thus, off-target activity can be determined easily, giving support to the medicinal chemists in their hit-to-lead and lead optimization studies.

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P618**Ensemble for Chemistry E-Notebook/Reaxys® Integration**Bjoern Loeprecht, Ismail Ijjaali

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MA 02140, USA

As electronic lab notebooks (ELNs) have become medicinal chemists' portal to the laboratory, having direct access to reaction databases from an ELN will allow medicinal chemists to both save time and increase productivity. To accomplish this, we have collaborated with Elsevier to provide a simple, effective way to search Reaxys direct from E-Notebook, and to import selected information from Reaxys into E-Notebook. The partnership between Elsevier and PerkinElmer ensures that Reaxys' best-in-class chemistry content from leading journals and patents is integrated with in-house, documented proprietary research findings via the Ensemble Notebook.

We have created an add-in in E-Notebook that adds a "Search Reaxys" button. When clicked, a search box appears in which a chemical structure or reaction can be drawn, pasted or selected from a reaction section, and additional search parameters can be added. When the search is submitted, the search parameters are sent to Reaxys via hypertext transfer protocol secure (https). Hits are displayed in Reaxys, and the scientist can use all of Reaxys' powerful navigation, sorting, and filtering tools to scan the hits and select any of interest. Once hits are selected, the scientist clicks a new "Export to ELN" button in Reaxys to send the data back to appropriate sections in E-Notebook.

P619**Parallel Fragment Screening and Docking against AmpC β -Lactamase**Sarah Barelrier, Oliv Eidam, Inbar Fish, Johan Hollander, Francis Figaroa, Ruta Nachane, Gregg Siegal, Brian Shoichet

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Experimental techniques like SPR and NMR are the methods of choice for fragment screening, although virtual screening has become more and more popular. In this first parallel study, we screened the same fragment library both virtually and experimentally to explore and compare the strengths and advantages of the methods. The ZoBio library (1443 compounds) was screened using the target-immobilized NMR screening (TINS) methodology against AmpC β -lactamase, a well-characterized therapeutic target.^[1,2] In parallel, the software DOCK was used to dock that same library against the enzyme, and the results of the two screenings were compared.^[3] Hits from both screens were then followed up by

enzymatic assay and X-ray crystallography, leading to several attractive starting points with interesting ligand efficiencies and physicochemical properties.

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P620

Rapid Purification of a Diverse Range of Peptides Using Flash Chromatography with ELSD and UV Detection

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Newly discovered peptide sequences are providing novel drug development candidates for use in modern medicines. Purification of natural products and synthetic peptides is an essential step in the drug discovery process and is typically accomplished using preparative chromatography, which can be expensive and time consuming. Flash chromatography is a fast and cost-efficient approach to purify synthetic peptides and other small molecules. Flash chromatography can quickly increase overall purity of peptides prior to the next amino acid addition or final polishing on preparative HPLC.

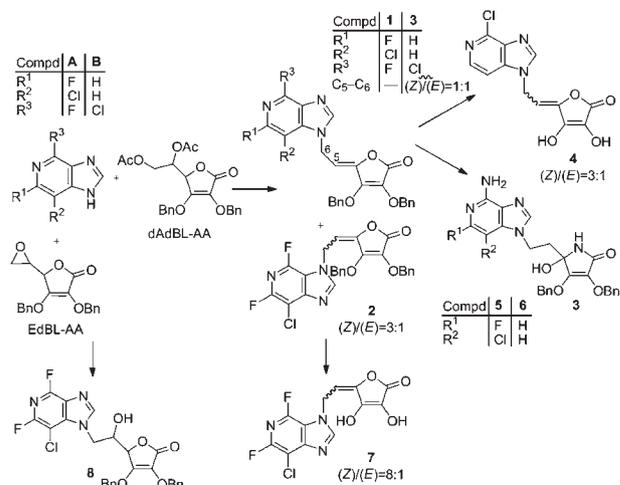
This work will review whether flash purification offers advantages for purifying a wide variety of peptides. The peptide samples range in size from a few amino acids up to 32 amino acids in length. Loading capacity is tested, as well as the ability of flash purification to resolve mixtures of peptides and small amino acids, and also partially purified reaction mixtures.

P621

Synthesis of Halogenated 3-Deazapurine and L-Ascorbic Acid Derivatives

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In research for antiviral and antitumor substances, many derivatives of natural nucleosides have been synthesized and used in the treatment of viral, tumor and bacterial diseases. Numerous synthesized 3-deazapurine derivatives have shown significant biological activity on a wide range of RNA and DNA viruses, a broad range of leukemia tumor cells lines and as antituberculostatics. In light of these findings and based on our previous study,^[1,2] we efficiently synthesized a series of new derivatives of halogenated 3-deazapurines (A, B) and L-ascorbic (1–4, 7–8) or imino-L-ascorbic acid (5–6) as a substitute for a sugar moiety to evaluate their antiviral and antitumor potency.

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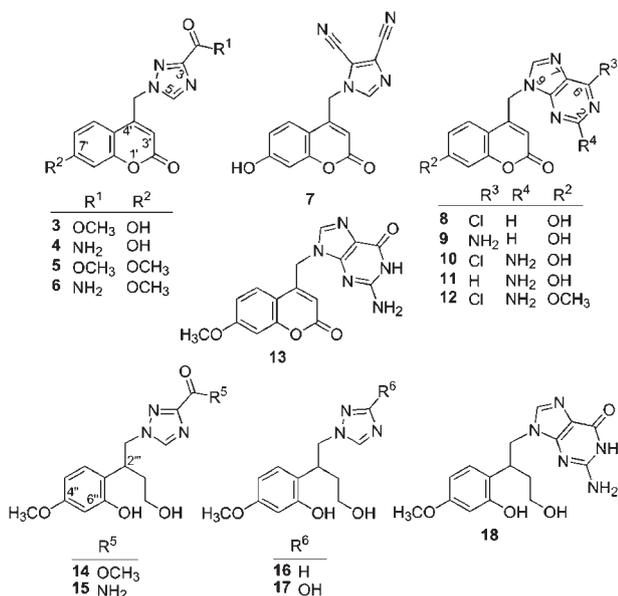
P622

Novel Coumarin Derivatives Containing 1,2,4-Triazole, 4,5-Dicyanoimidazole and Purine Moiety: Synthesis and Evaluation of Cytostatic Activity

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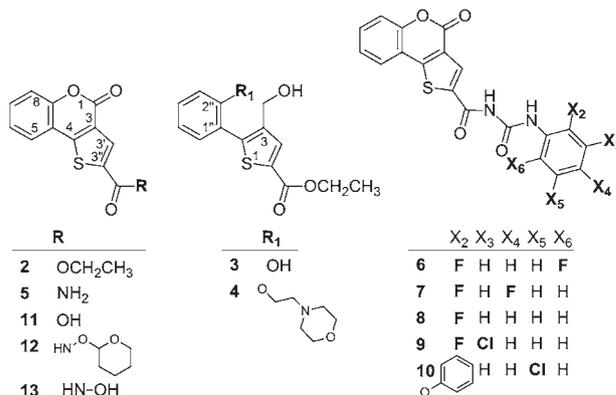
Here, we report the synthesis and in vitro antitumor effects of a series of novel 1,2,4-triazole (**3–6**), 4,5-dicyanoimidazole (**7**), and purine (**8–13**) coumarin derivatives and their acyclic nucleoside analogues (**14–18**). The structures of novel compounds **3–18** were deduced from their ¹H and ¹³C NMR data and corresponding mass spectra. Results of an antiproliferative assay performed on a panel of selected human tumor cell lines revealed that compound **6** has moderate cytostatic activity against the HeLa cell line (IC₅₀=35 μM), whereas compound **10** shows moderate activity against HeLa (IC₅₀=33 μM), HepG2 (IC₅₀=25 μM) and SW620 (IC₅₀=35 μM) cell lines. These compounds exhibited no cytotoxic effect on normal (diploid) human fibroblasts.

P623

Synthesis of Novel Ureido and Hydroxamic Derivatives of Coumarin[4,3-c]thiophene

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Aromatic urea derivatives have been widely used for cancer patients with great clinical success and are continuing to be important chemotherapeutic agents. This class of compounds has been proven to be tubulin ligands that inhibit the polymerization of tubulin. We synthesized a series of novel ureido (**6b–10b**) and hydroxamic (**12b–13b**) derivatives of coumarin[4,3-c]thiophene for their antitumor and antiviral activity evaluation.

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