Plenary, Award and Prize Lectures

PL001 | The Ubiquitin Proteolytic System—From Basic Mechanisms through Human Diseases and on to Drug Development

Aaron Ciechanover

Cancer & Vascular Biology Research Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Between the 50s and 80s, most studies in biomedicine focused on the central dogma—the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a nonspecific, dead-end process. While it was known that proteins do turn over, the high specificity of the process—where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared—was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes, such as cell cycle and differentiation, communication of the cell with the extracellular environment, and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies, and neurodegeneration among them, and that the system has become a major platform for drug targeting.

PL002 | Innovation to Create Value for Patients

Ismail Kola

UCB, Allée de la Recherche 60, 1070 Brussels, Belgium

Our industry is at an evolutionary inflection point, with patent cliffs, R&D productivity challenges and increasing demand for value from all customer groups requiring new approaches in order to thrive. A strategy for successful medicines of the future will centre on pipelines of true breakthroughs rather than incremental innovation, to meet patient need whilst achieving reimbursement. We must leverage recent scientific advance to better understand defined patient populations and robustly mitigate attrition risk, whilst operating as part of a holistic global network to maximise scientific strengths and new discoveries. This talk will explore these themes and the science that drives them.

PL003 | Medicinal Chemistry Experts in Pharma Are Helping Us To Generate Freely Available Novel Probes in Order To Facilitate Target Discovery

Chas Bountra

SGC, Department of Clinical Medicine, University of Oxford, UK

Despite large investments by governments, charities and the private sector, “pioneering” new medicines remain scientifically challenging and costly to produce. With a price tag of about £1Bn for each new medicine and with many drug discovery programmes failing in early clinical studies, we need to take a different approach. There is a huge opportunity for improving our success rate by minimising parallel efforts in early phases across academia, biotech and pharma.

Several public, charitable and private funders (currently 9 large pharmaceutical companies) have pooled their resources and expertise to establish the SGC. This “public private partnership” (PPP) based at the Universities of Oxford and Toronto is generating novel, freely available reagents for drug discovery. The group works closely with a network of more than 250 academic labs across the world, to exploit these reagents in basic science and drug discovery. All findings are rapidly disseminated.

The impact of the SGC has been profound. Working together we have solved 25 % of all human protein structures. We are now creating small molecule probes; “prototypic medicines” which are made freely available to help all partners and scientists across the world, understand the mechanisms underlying human diseases. These pre-competitive efforts have resulted in new proprietary programmes in pharma, the establishment of new biotechs, and science on a grand scale.

We are now aiming to build human disease platforms, to more systematically profile novel probes in human control/disease/IPS cells, using functional, medium-throughput readouts. We believe such platforms will allow direct comparison of probes/targets on different cell types, from different disease cohorts, and thereby enable better target discovery.

Since, “definitive” target validation occurs in patients, we are now joining forces to progress pioneers targets into phase II studies as quickly as possible. We will also do this as a pre-competitive PPP.

The generation of novel probes and clinical molecules for evaluation in patients, is and will require access to a very large number of skilled medicinal chemists.

PL004 | Abstract unavailable at the time of printing

PL005 | Opening the Future: Individualized Medicinal Systems Chemistry

Hans V. Westerhoff and friends

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2) Synthetic Systems Biology, SILS, NISB, the University of Amsterdam, The Netherlands
3) Manchester Centre for Integrative Systems Biology, University of Manchester, UK

Even after days of hearing about exciting progress in medicinal chemistry, we may still realize that when applied to an individual patient, most drugs do not work as well as anticipated. Of course we know why: the assignment is to cure living organisms, not a macromolecule. *Their* illnesses are systems biology diseases, i.e., malfunctionings of the networks that produce function. *Their* macromolecules are like moving targets; the moment one hits them, adaptation or selection induces overexpression or parallel pathways. The question is what we do about this problem, i.e., look the other way, or engage in network-based drug target identification and drug design. For the case of anti-trypanosomal drugs, I will exemplify the shift to such a systems-biology approach where new targets are identified, where the drug affects parasite more than host, where the target seems to move our way quasi-suicidally, and where new molecular requirements and new medicinal chemistry become evident.

For safety and against quackery, diagnosis, medicines, therapies and their validation have been standardized: One drug for all. Yet, any two individuals differ widely. Because of lack of information on the differences, we need to accept the fog of statistics: We test drugs in clinical trials accepting the paradigm that any individual stands a 40% chance of being cured by the drug. But here, the Einstein–Bohr discussion returns. We may not really like to throw dice when administering drugs to patients: The statistical uncertainty reflects lack of knowledge, which we may be able to redress if not in quantum mechanics, then at least in medicinal chemistry and medicine. If we were to have more information and understand the molecule-based physiology of patient and disease, then the effect of a drug should be predictable and statistics unnecessary. With such understanding, the chance that the drug works for any particular patient should be >90 %.

It has become completely feasible to know precisely the genome sequence of any diseased individual, as well as transcriptome, proteome and metabolome of certain tissues. Lacking was the step from the knowledge of genomics to the understanding of the molecule-based physiology of patient and disease. This is what systems biology begins to add. Recon2, the May 2013 consensus reconstruction of genome-wide human metabolism, enables the generation of a metabolic map and of more lively models, for each sequenced individual. I will exemplify this for individualized biomarking of glutathione-based drug detoxification capacity and perhaps for an approach against chronic inflammation.

Conjunction of such a genomics-driven approach with an equally individualized physiological, life-style approach may lead to new strategies for truly individualized medicinal chemistry: More and better chemicals for better individualized therapies. I will discuss implications for development, assessment, trialing and renewed certification, but then in a systems-biology assisted way, of individualized cocktails of revived blockbusters.

AL001 | Pursuing Compound Quality

Paul Leeson

GlaxoSmithKline, UK

A significant body of meta data emphasises the importance to drug discovery strategies of reducing ADMET risk by optimising the molecular properties of hits, leads and drug candidates.[1,2] Concern for the health of pharmaceutical pipelines stems from analyses of molecules patented by the industry, which are on average more lipophilic and larger than approved oral drugs.[2,3] This issue is highly relevant to pipeline attrition, where only ~4 % of candidate drugs reach the market and compound-related risks, in dose, exposure and toxicity, have often been carried from discovery into more costly clinical development.[4] Compound quality is controllable, being fixed at the point of design, and varies substantially across organisations.[3] Improving compound quality can be facilitated, inter alia, by selection of lead-like chemical starting points[5] and using ligand efficiency measures[2,6] to guide optimisation.

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AL002 | Small-Molecule Control of Intracellular Protein Levels

Craig M. Crews

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While consisting of successful drugs, the current pharmacopeia has inherent limitations based on its 'Occupancy-based' paradigm of pharmaceutical control. These limitations include: 1) the need to achieve/maintain high systemic exposure to insure sufficient in vivo protein inhibition, 2) the potential off-target side effects due to high in vivo concentrations, and 3) the need to bind to an active site, thus limiting the potential 'drug target space' to a fraction of the proteome. As an alternative pharmaceutical strategy, induced protein degradation lacks these limitations. Based on an 'Event-driven' paradigm, this approach offers a novel, catalytic mechanism to irreversibly inhibit protein function, namely, the intracellular destruction of target proteins. This is achieved via recruitment of target proteins to the cellular quality control machinery, i.e., the Ubiquitin/Proteasome System (UPS). For the past two decades, the Crews lab has focused on developing different strategies for inhibiting (via proteasome inhibitors) or inducing protein degradation, including the Proteolytic Targeting Chimera (PROTAC) and Hydrophobic Tagging (HyT) technologies. These latter heterodimeric ligand approaches offer the potential to selectively knock down intracellular levels of specific proteins, irrespective of protein class, thus allowing one to target those proteins that are currently not '*pharmacologically vulnerable*'.

AL003 | Thermodynamics-Assisted Drug Discovery

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Optimization of compounds to drug candidates requires a simultaneous improvement of potency, selectivity and pharmacokinetic (PK) profile, the latter is efficiently characterized by physicochemical properties. It has been, however, recognised that potency increase is most often accompanied by increasing molecular weight and lipophilicity that adversely affect PK. Analysis of available thermodynamic profiles of drugs and drug candidates revealed that the enthalpy and entropy content of ligand–protein binding exhibit characteristic size and potency dependence. The binding of small ligands is typically enthalpy dominated while entropy contribution inevitably increases with ligand size. This observation is in line with the relation of ligand size and dominant interactions of ligand–protein binding. Small molecules most often bind with few optimal H-bonds that contribute to enthalpy lowering, while the driving force of binding for larger ligands is apolar desolvation that primarily contributes to beneficial entropy changes. This explains why drug discovery optimizations tend to result in mostly large and apolar compounds that bind to their target with high affinity but have suboptimal PK properties. Binding thermodynamic considerations offer a balance between potency and physicochemical property inflation that can be achieved by monitoring binding thermodynamics in medicinal chemistry optimizations. Potency and thermodynamic profile can be well characterized by ligand efficiency metrics (like LE, LLE, LELP) that are obtained from potencies and from calculated physicochemical data. Ligand efficiency metrics are beneficial indicators of the balanced properties of ligands and their optimized values are good indicators of compound quality. They can replace thermodynamic measurements at a large extent while they allow the application of binding thermodynamics revealed rules to produce better quality drug candidates.

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AL004 | Medicinal Chemistry, Quo Vadis? The Changing Climate in R&D

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Today's innovative drug discovery is costly and time consuming, with very few novel therapeutics making it to the market. The enterprise of drug discovery and development is fundamentally shifting in the last decade. Dramatic and irreversible changes are reshaping the roles of the pharmaceutical, biotechnology and academic areas and consequently also the role medicinal chemistry.

Over the past decade, scientific and business needs have driven the pharmaceutical industry to more closely align drug discovery and drug development efforts. The general view is that the process whereby future drugs are discovered and developed will be fundamentally different to how these activities were performed in the past.

Traditional medicinal chemistry approaches adopted during the 1970s and 1980s were focused primarily on analoguing of endogenous ligands and industry leads, moving from in vivo models to a single target selective drug for a single mechanism. Following the lock and key model proposed by Ehrlich more than a century ago, over the previous decades, drug discovery efforts have focused on

identifying single selective drugs that target a single mechanism; that is, identifying ligands ('keys') that fit into specific targets ('locks'). Achieving target specificity of active compounds has, for at least three decades, been considered a hallmark of drug discovery efforts, as a consequence of an increasingly sophisticated molecular approach to discovery, primarily focusing on isolated targets and specific binding assays.

Chemistry was low throughput and done iteratively, driven primarily by biochemical observations derived from animal testing. In contrast, the past decade has witnessed an evolution in medicinal chemistry approaches wherein automation has been effectively utilized in the synthesis of large numbers of analogues (combinatorial chemistry) and in the rapid screening of large numbers of compounds (HTS). At the turn of the century, subsequent to identification and characterization of large numbers of targets, the deciphering of the human genome led to an explosion in the "-omics" technologies. The assimilation of the resulting information and correlation of potential therapeutic targets with human diseases present tremendous challenges for drug research.

The development of multitarget drugs experienced a renaissance during the past decade. Small molecules which interact with more than one target seem to be a very attractive to address various diseases. The postulated advantages include better efficacy coming along with less unwanted side effects and a simpler pharmacokinetic profile compared to a combination of two or more selective drugs.

In addition, medicinal chemists now routinely impart a targeted effort to incorporate drug-like properties into their chemical scaffolds, with the prevailing goal of not only identifying a compound that can advance into development, but also identifying an associated solid state phase that has appropriate physicochemical characteristics that allow for optimal in vivo performance. A recent trend in drug discovery has been to view the process of drug design as a multi parameter optimisation problem, in which, from the beginning, a project team attempts to identify drug candidates that achieve an optimal balance of the biological and physicochemical properties required for a chosen therapeutic objective.

PR001 | Phage Selection of Bicyclic Peptides for Therapeutic Application

Christian Heinis

Ecole Polytechnique Federale de Lausanne (EPFL), Institute of Chemical Sciences and Engineering, Lausanne, Switzerland

My laboratory is engaged in the discovery and development of new peptide formats for the use in therapy. A major focus is the development of bicyclic peptide ligands of disease targets using a combinatorial approach based on phage display (see Figure).[1] The bicyclic peptides combine key qualities of antibody therapeutics (high affinity and specificity) and advantages of small-molecule drugs (access to chemical synthesis, diffusion into tissue, various administration options). We were able to generate bicyclic peptide antagonists or ligands with nanomolar or even picomolar binding affinity to a range of human disease targets including plasma kallikrein, urokinase-type plasminogen activator, coagulation factor XIIa, matrix metalloproteinase 2 and sortase A.

Towards the therapeutic application of the peptides, we have extended their circulation time to several days in mice, and we are now assessing the therapeutic effect of some of the peptides in vivo. An important activity of my research group is also the development of novel peptide macrocycle formats with even better binding properties.[2–4]

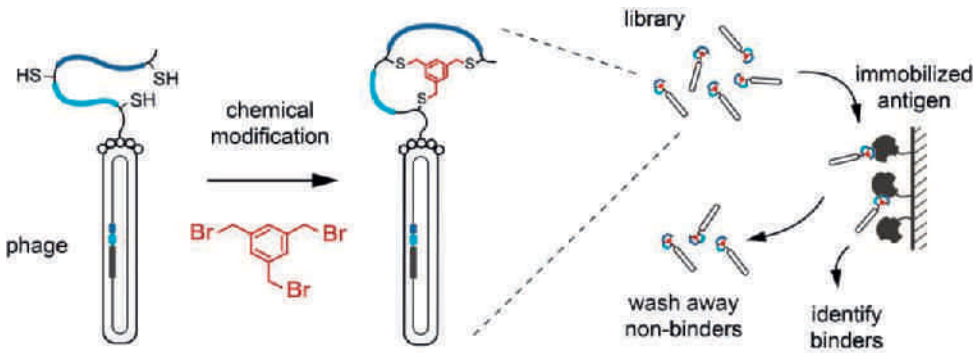

Figure. A) Large libraries of random peptides (>4 billion different peptides) are displayed on phage and cyclised in a chemical reaction (left). Binders to targets of interest are subsequently isolated in affinity selections (right); B) Chemical structure of an isolated bicyclic peptide.

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PR002 | Design, Synthesis and Properties of Potent Inhibitors of *Pseudomonas aeruginosa* Deacetylase LpxC

Grazia Piizzi, David Parker, Markus Dobler, Som Wattanasin, Xia Yang

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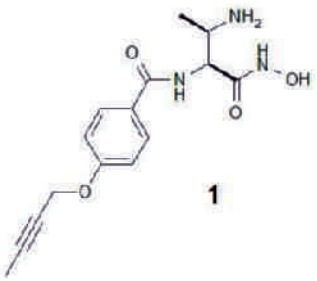
Over the past several decades the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rates. This problem is compounded by the existence of bacterial strains resistant to multiple antibacterials. *Pseudomonas aeruginosa* is a leading cause of nosocomial infections, and its resistant strains survive to virtually all approved antibacterials. Herein we describe our approach to inhibit the deacetylation of uridyldiphospho-3-*O*-(*R*-hydroxydecanoyl)-*N*-acetylglucos-amine by LpxC. This is the first committed step in the biosynthesis of lipid A of Gram-negative bacteria, including *Pseudomonas aeruginosa*. Design, synthesis and biological evaluation of several potent hydroxamic acids, such as **1**, will be discussed.



1

***P. aer.* LpxC IC_{50} = 8.7 nM**
MIC wt *P. aer.* = 2.0 ug/mL

PR003 | Inhibitors and Substrates of the Human Lysine Deacylase Enzymes

Christian Adam Olsen

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A brief introduction to the histone deacetylase (HDAC) enzymes, or perhaps more appropriately the lysine deacylase (KDAC) enzymes, will be given. A few of our results related to substrate specificity will be discussed and the majority of lecture will then be devoted to work on macrocyclic peptide-based inhibitors of this class of enzymes. Several cyclic tetrapeptide and depsipeptide natural products have proven useful as biological probes and drug candidates due to their potent activities as HDAC inhibitors. Here, the synthesis of such a class of cyclic tetrapeptide HDAC inhibitors, the azumamides, will be presented. Our concise synthetic route, in which the key step for preparation of a non-canonical disubstituted β-amino acid building block was an Ellman-type Mannich reaction, furnished the natural products as well as structurally modified analogues. These efforts delivered azumamides B–D through total synthesis for the first time, which corroborated the originally assigned structures and enabled full profiling of the HDAC inhibitory properties of the entire selection of azumamides A–E. Furthermore, evaluation of an extended series of analogues containing various structural modifications, by HDAC profiling, NMR structure determination, and molecular docking to HDAC crystal structures revealed insight into the requirements for potent HDAC inhibition by macrocyclic peptides.

Lectures

LE001 | Inflammation, Obesity and Metabolic Disease

Ajay Chawla

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Vertebrate tissues comprise precise admixtures of parenchymal and hematopoietic cells, whose interaction is vital to proper tissue function. By regulating this interaction, vertebrates are able to mitigate environmental stress and coordinate dramatic physiologic adaptations. White adipose-tissue-associated macrophages, for example, are necessary for proper systemic insulin signaling and brown/beige fat thermogenesis. Under conditions of chronic nutrient excess, adipose tissue macrophage recruitment and activation increase in an effort to decrease excess nutrient storage and alleviate adipocyte stress. While basal equilibria may be re-established upon normalization of nutrient intake, a new set point characterized by insulin resistance and chronic inflammation is established if the stress persists. While this response is adaptive in acute overfeeding and infection, its application in the modern context of obesity can have catastrophic health consequences. Understanding the molecular basis for how leukocyte set points are established, maintained, and regulated in tissues is thus critical to our understanding of and intervention in chronic metabolic diseases. At this meeting, I will discuss the molecular and cellular mechanisms that link inflammation to obesity and metabolic disease, and provide insights into pathways that potentially can be targeted for the treatment of obesity, insulin resistance, and type 2 diabetes.

LE002 | CB_2 Agonists Protect from Inflammation-Related Damage and Fibrosis

Uwe Grether, Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Fingerle, Ivan Formentini, Jürgen Funk, Sabine Grüner, Atsushi Kimbara, Matthias Nettekoven, Giorgio Ottaviani, Camille Perret, Mark Rogers-Evans, Stephan Röver, Franz Schuler, Tanja Schulz-Gasch , Christoph Ullmer

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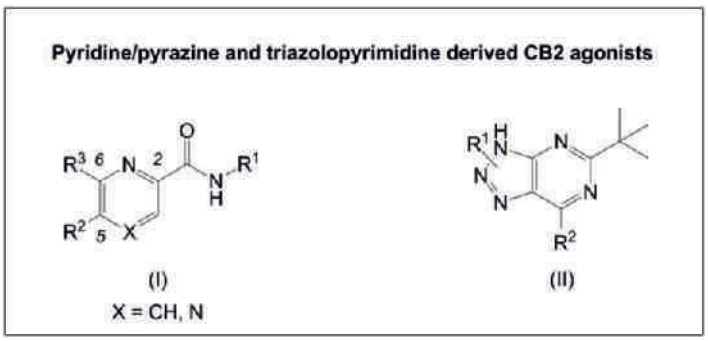
Agonists of the cannabinoid receptor 2 (CB_2) positively influence a large number of pathological conditions, spanning from cardiovascular, gastrointestinal, liver, kidney, lung, neurodegenerative and psychiatric disorders to pain, cancer, bone, reproductive and skin pathologies.[1] CB_2 agonists have been shown to exhibit protective effects in several animal models after inducing ischemia in organs like liver and brain. Furthermore, CB_2 agonists were also shown to protect organs such as liver from fibrosis. 2,5,6-Trisubstituted pyridines and pyrazines (**I**) as well as triazolopyrimidines (**II**) were identified to be novel, highly potent and selective CB_2 agonists. The exploration of the different exit vectors led to the discovery of agonists with CB_2 picomolar potency in inhibiting cAMP formation. Surprisingly, only subtle structural changes inverted the function from agonistic into inverse agonists. Furthermore, species differences between human and rodent CB_2 receptors have been observed. A detailed structure activity relationship for CB_2 and CB_1 binding and functional assays was elaborated.



Physicochemical properties including solubility, membrane permeation and lipophilicity as well as metabolic stability and cytochrome P450 inhibition potential were optimized. Advanced compounds combined high in vitro potency with favorable early ADME properties and have been profiled in in vivo pharmacokinetic and efficacy studies. Selected compounds from both series such as RO6839828 and RO6871304 were found to protect mouse kidneys from ischemia reperfusion injury. After a period of 25 min ischemia @ 37 °C and 24 h reperfusion, a statistically significant improvement in kidney function as measured by plasma creatinine levels was produced. Moreover, the efficacy was reflected in the reduction of relevant plasma biomarkers of kidney injury (NGAL, osteopontin). In addition, both compounds significantly reduced fibrosis in a rat unilateral ureter obstruction model (UUO) as measured by a reduction in collagen I deposition 8 days after UUO, thereby suggesting that CB_2 agonists might have beneficial effects in both acute and chronic kidney disease.

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LE003 | The Discovery of APD334, A Selective $S1P_1$ Functional Antagonist

Robert M. Jones

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APD334 is a NCE being developed by Arena Pharmaceuticals for the treatment of relapsing–remitting forms of multiple sclerosis (RRMS). It is an orally available, selective, sphingosine 1-phosphate subtype 1 receptor ($S1P_1$) functional antagonist. Sequestration of T-lymphocytes into lymph nodes and other secondary lymphoid tissues by $S1P_1$ receptor functional antagonists has been of therapeutic interest for the treatment of a variety of autoimmune diseases. In particular, the approved oral pan-S1PR modulator Gilenya (fingolimod) has been shown to reduce the frequency of clinical exacerbations and to delay the accumulation of physical disability in RRMS. More selective $S1P_1$ compounds that may have reduced side effect profiles have thus become highly sought after. Herein, we will highlight the design and synthesis of a second-generation series of orally efficacious small-molecule $S1P_1$ functional antagonists that culminated in the identification of the clinical candidate, APD334.

LE004 | Discovery of Small-Molecule RORγ Modulators for the Treatment of Autoimmune Diseases

Frank Narjes, Yafeng Xue, Johan Jirholt, Stefan von Berg, Agnes Leffler, Hanna Grindebacke, Eva Hansson, Jane McPheat, Roine Olsson, Matti Lepistö, Antonio Llinas, Thomas G. Hansson

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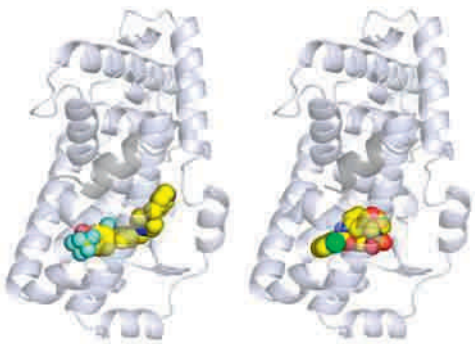
Aberrant activity of pro-inflammatory T_H 17 cells leading to the overproduction of the cytokine interleukin (IL)-17 has been suggested to play a role in the pathology of autoimmune diseases including rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease.[1] Recent positive results from clinical trials of Secukinumab and Brodalumab, two monoclonal antibodies against IL-17A or IL-17 receptor A, in patients with psoriasis or psoriatic arthritis highlight the importance of IL-17 in inflammation.[2]

The nuclear receptor RORγ t, an isoform of the retinoic acid-related orphan receptor gamma (RORc, RORγ), is exclusively expressed in cells of the immune system and was shown to be the master regulator for function and development of T_H 17 cells. Apart from the modulation of IL-17, RORγ also modulates the expression of other pro-inflammatory stimuli such as IL-21, IL-22 and indirectly granulocyte-macrophage colony stimulating factor. Inhibition of RORγ activity with a small-molecule ligand could therefore present an attractive and broader option with respect to biologics for the treatment of autoimmune diseases. Preliminary studies with knock-out mice, as well as experiments with small-molecule inhibitors of RORγ in animal models, support this notion. Additionally, there is accumulating evidence that RORγ signaling may play a role in metabolic disorders.[3]



Here, we describe our own hit-to-lead effort to identify modulators of this receptor. Through focused screening of our compound collection, we identified several series of molecules, which displayed micromolar affinity for the RORγ ligand binding domain (LDB) in a radioligand binding assay format. Optimization of these initial hits resulted in potent binders, which dose-dependently decreased the ability of the RORγ-LDB to interact with the steroid receptor co-activator peptide 1. From the same chemical series, agonists with similar binding affinity were also identified. The best inverse agonists inhibited the release of IL-17 secretion from isolated and cultured human T_H 17 cells. Cocrystal structures of inhibitors bound to the RORγ LDB were obtained, and we will discuss the binding mode in comparison to crystal structures of other known RORγ modulators.

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LE005 | Recent Advances in Asymmetric Catalysis

Erick Carreira

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The ability to readily access small-molecule building blocks at will has important consequences for the discovery and development of novel medicines and materials. It is particularly beneficial when the chemical methods are convenient while at the same time economically and environmentally tenable and sustainable. A focus of our research program at ETH-Zurich is the identification, study, and development of novel reactions and methods for preparation of functionalized structures. We are especially interested in catalytic processes that are easily executed and utilize readily available starting materials. We will discuss several new reaction processes that provide ready access to a host of fundamentally versatile building blocks for synthesis. The presentation focuses on the unique reactivity of Ir-complexes with a novel phosphoramidite-olefin ligand. We have found that these can activate allylic alcohols towards a wide range of direct displacement reactions, giving rise to optically active products.

LE006 | New Stereoselective Organocatalytic Reactions for the Synthesis of Isoquinoline Alkaloids

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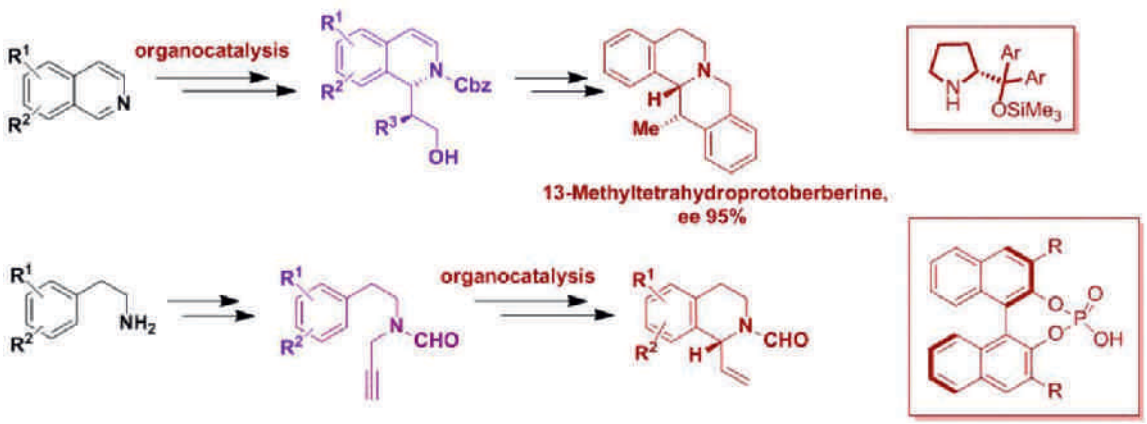
13-Methyl tetrahydroprotoberberine alkaloids and related compounds are a group of tetrahydroprotoberberine alkaloids having an extra methyl or alkyl substituted group at the C-13 position. They are the major active constituents of Corydalis species and they show significant cytotoxicity and therapeutic effects. Although a few stereoselective synthetic approaches have been described for the class of tetrahydroprotoberberine alkaloids, catalytic stereoselective methodologies towards 13-alkyl tetrahydroprotoberberine alkaloids, to the best of our knowledge, were not disclosed yet.[1] We present here an organocatalytic highly sterereoselective addition of aldehydes promoted by the Hayashi-Jørgensen secondary amine catalyst to isoquinoline by the use of CbzCl or Boc_2O to activate isoquinoline towards the addition. This procedure is quite general, gives high enantiomeric excesses, and allows the simple deprotection of the useful synthetic intermediates. The first enantioselective synthesis of a 13-methyl tetrahydroprotoberberine alkaloid, through a new, simple and rapid approach is reported, with the compound showing interesting anticancer properties. Furthermore, a Brønsted acid catalyzed approach towards enantioenriched intermediates for the synthesis of isoquinoline will also be illustrated.



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LE007 | Metalation of Polyfunctional Heterocyclic Compounds: Applications in Agrochemistry

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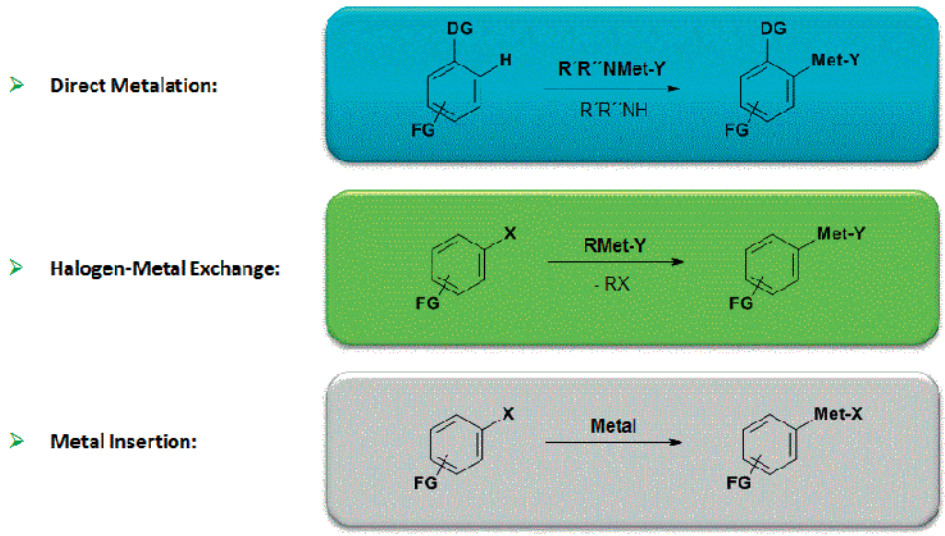
Metalation is an important tool for the functionalization of aromatic and heteroaromatic rings and has already found many applications in the pharmaceutical and agrochemical industry.[1]

Within the last years, several new approaches in the area of carbon-hydrogen activation (use of sterically hindered bases for directed ortho metalation) or carbon-halogen activation (halogen/metal exchange or direct metal insertion) have been developed.[2] Lithium, magnesium and zinc organometallics have proven to be excellent reagents for the preparation of highly functionalized heteroaromatics. Their features will be discussed and illustrated especially in the case of directed *ortho* metalations,[3] with examples taken from both academia and industry.[4] Finally, future perspectives will be presented in the field of C-H activation, as exemplified by the development of new sterically hindered organometallic bases.[5]

Metal–Carbon Bond Formation



FG: Functional Group

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LE008 | Development of Novel Building Blocks to Accelerate Drug Discovery

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Structural diversity of the known drug-like compounds is very low. Among all possible structures with less than 10 heavy atoms, only a tiny part has been synthesized in reality. The same is true for medicinal chemistry projects: one still uses only the common building blocks, while many others remain to be explored. It's known that even small structural changes can lead to drastic changes in activity and selectivity. Therefore, medicinal chemists need a library of isomeric/homologous building blocks to efficiently fine-tune the "hit" structure. In this work, we synthesized several novel structural motifs—analogues of already established structures. Details of the design, synthesis and application of these molecules will be discussed.

LE009 | Mechanoenzymatics of Biomolecular Machines: Free Energies, Dynamics, Function

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Proteins are biological nanomachines. Virtually every function in the cell is carried out by proteins, ranging from protein synthesis, ATP synthesis, molecular binding and recognition, selective transport, sensor functions, mechanical stability, and many more. Over the past years, the combined interdisciplinary efforts—specifically, atomic force probe experiments, spectroscopy, and atomistic simulations—have revealed how many of these functions are effected on the molecular level.[1–6,9,10] Key to our understanding is an intimate connection between forces and enzymatic activity. As these machanoenzymatic processes are challenging to access experimentally, atomistic molecular dynamics simulations play a pivotal role in this enterprise, as they offer both unparalleled temporal and spatial resolution. This talk will illustrate the type of questions that can (and cannot) be addressed, and its (current) limitations. The examples include mechanics of energy conversion in F-ATP synthase,[4,5,10] the mechanical properties of a phage connector[2] and of viral capsids,[6] as well as tRNA translocation within the ribosome.[9] We will further demonstrate how atomistic simulations enable one to mimic, one-to-one, single-molecule experiments such as FRET distance measurements, and thereby to enhance their accuracy.[7] We will, finally, take a more global view on the 'universe' of protein dynamics motion patterns and demonstrate that a systematic coverage of this 'dynasome' allows to predict protein function more reliably than purely structure-based methods.[8]

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LE010 | Efficient Determination of Protein–Ligand Standard Binding Free Energies and Permeabilities with a Computational Microcalorimeter

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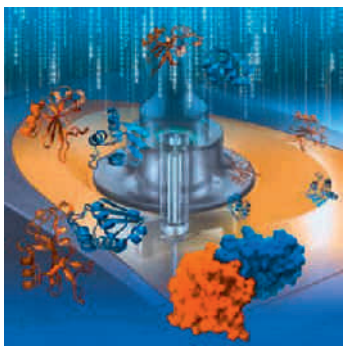
One of the grand challenges of rationale drug discovery is the prediction of the affinity of potential therapeutic agents for a given protein target. This challenge is rooted to a large extent in the considerable changes in configurational entropy underlying the binding process that atomistic simulations cannot easily sample. Two strategies relying upon alchemical transformations and potentials of mean force are proposed, invoking a series of geometrical restraints acting on collective variables designed to alleviate sampling limitations inherent to classical molecular dynamics simulations. Downstream from the prediction of binding affinities is the equally challenging prediction of bioavailability. To estimate the permeability of the biological membrane to a drug candidate, an approach based upon Bayesian inferences, which reconciles thermodynamics and kinetics in molecular dynamics simulations with time-dependent biases, is put forth.



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LE011 | Binding Affinity Prediction from Molecular Simulation—Soon a Standard Method in Structure-Based Drug Design?

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Affinity to the target of interest is a crucial optimization parameter in early drug discovery. Being able to reliably predict protein–ligand binding affinity would enable us to restrict synthesis to the most promising compounds. In silico profiling of compounds prior to synthesis is routinely done for many optimization parameters such as physicochemical properties or ADMET-related endpoints. However, binding affinity prediction has so far been absent from the list of predicted properties. Equations that allow rigorous calculation of protein-ligand free energy of binding have been formulated decades ago.[1–3] These physics-based methods rely on molecular simulation and allow an estimation of the binding affinity which includes all entropic and enthalpic effects. In practice these methods are limited by the accuracy of the potential energy function, the so called “force-field”, used to describe inter- and intramolecular interactions and the extent to which the conformational space is sampled in the given simulation time. Furthermore, the tedious setup of such calculations as well as long calculation times has so far hampered large-scale application in industry. Several recent developments may change this, however. Multiple schemes for automated setup of these calculations have been introduced recently.[4–6] GPU-based simulations now allow sufficient sampling within reasonable time and allow computations to match project timelines. We present the results of an extensive evaluation of a recently developed software solution[7,8] that combines a state-of-the-art condensed phase force-field for arbitrary organic molecules,[9] enhanced sampling techniques, statistically sound free energy estimators and makes use of the speed of GPU-based molecular simulations.

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LE012 | P-Glycoprotein/Membrane Role on Multidrug Resistance: Insights from In Silico Studies

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Multidrug resistance (MDR) to anticancer drugs is currently a serious health concern due to the increase number of chemotherapy failures reported worldwide. Since the expression of ABC transporters is frequently increased in many cancers and tumor cell lines,[1] the inhibition of ABC efflux pumps, such as P-glycoprotein (P-gp), is considered a promising approach for overcoming MDR.

Following our characterization of three drug-binding sites within P-gp's internal drug-binding pocket (DBP), a series of molecular dynamics studies are undergoing to further clarify the impact that the lipid bilayer and the transporter itself have in promoting drug efflux. Previous free energy profiles for molecule transfer from the lipid bilayer into the water bulk environment[2] revealed a greater propensity for efflux modulators to permeate into the membrane when compared to substrates like colchicine and vinblastine. When taking into account protonation (as in tariquidar), the difference between the obtained curves seem to suggest that lipid head groups may have a strong influence on membrane partition by controlling molecules' protonation/deprotonation rate.[3] On the other hand, molecule transfer from the membrane's hydrophobic environment into the water-filled DBP revealed a lower energetic cost for substrate permeation when compared with the modulator tariquidar. These results, together with the ones described above, corroborates that the concentration inside the membrane is a key factor that may affect modulation capability.

The possible influence of P-gp's nucleotide-binding domains (NBDs) on the drug's adsorption free energy[4] was also assessed, suggesting that P-gp's cytoplasmic domains specifically increases local concentration for some molecules, which may promote higher

permeation rates towards the lipid bilayer. In addition, Normal Mode Analysis[5] suggests that molecules may induce different NBD motion patterns, altering NBDs dimerization with direct consequences on conformational changes during efflux.

Acknowledgements:

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LE013 | Natural Products as Driving Force for Innovation in Pharmaceutical Research and Development

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Natural products are evolutionarily designed and chemically distinct from most synthetic library molecules. Today, they are complementary to other drug discovery technologies and have a proven track record in phenotypic screens. An array of target identification technologies have been developed to study the mode of action of these molecules, which have been optimized through evolution for their biological activities. The Novartis genome mining project has confirmed that the great potential of bacteria and filamentous fungi as a source for new chemotypes has not been fully exploited. Secondary metabolomics and genetic engineering can be used to uncover the true biosynthetic potential of a strain. A further step towards an increased accessibility of natural products from diverse sources is the printing of entire biosynthetic pathways combined with heterologous expression. Synthetic biology concepts and technologies will shape the future of natural product research.

LE014 | Sponging Off Nature for New Drug Leads

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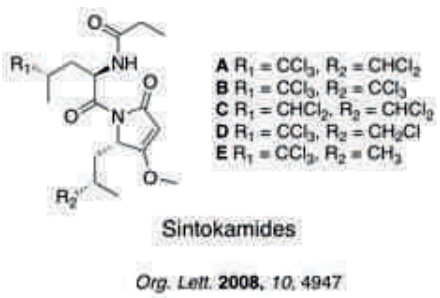
The secondary metabolites found in marine organisms represent an extremely rich source of novel chemical diversity for drug discovery and chemical biology programs. Among the marine invertebrates, marine sponges continue to be the most prolific source of new natural products. Our group at UBC has amassed a sizable library of crude extracts from marine sponges collected in many of the world's oceans. In collaboration with biologists, this crude extract library has been screened for activity in a variety of cell-based and pure enzyme assays designed to identify promising lead compounds for the development of drugs. Bioassay-guided fractionation of the crude extracts and extensive spectroscopic analysis is used to identify the structures of the pure natural products active in these assays. Biology-oriented chemical synthesis is used by our group to probe the SAR for new natural product pharmacophores that we discover and to provide material for in vivo testing in animal models. This lecture will focus on the chemistry and biology of the sintokamides, a family of chlorinated peptides that we discovered in the sponge *Dysidea* sp. collected in Indonesia. We have shown that the sintokamides are promising lead compounds for the development of drugs to treat castration recurrent prostate cancer (CRPC) and we have carried out an extensive program of analogue synthesis in order to generate SAR information about the anticancer pharmacophore for this family and to generate probes to identify their molecular target and mechanism of action.



LE015 | Bioactive Secondary Metabolites from the Soil and Marine-Derived Fungi of the Genus *Neosartorya*

Anake Kijjoa

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Neosartorya species are sexual form of the *Aspergillus* species, notably the section *Fumigati*. Except for *N. pseudofisheri* which is implicated in several illnesses such as osteomyelitis,[1] pulmonary infection[2] and peritonitis,[3] other species of this genus have not been widely investigated, especially for their secondary metabolites. For this reason we have investigated the secondary metabolites of the cultures of several species of the soil and marine-derived fungi of this genus in order to compare their chemical profiles as well as to evaluate their biological activities. We now report the results of our chemical investigation of the culture of the soil-derived *N. glabra*, *N. pseudofischeri, N. siamensis, N. fischeri,* and the marine-derived *N. paulistensis*, *N. laciniosa* and *N. tsunodae,* as well as the in vitro antitumor activity on human tumor cell lines, and the antibacterial and antibiofilm activities against the multidrug-resistant strains of the secondary metabolites isolated from these fungi.[4–8]

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LE016 | From Malaysian Biodiversity to the Total Synthesis of Dual Inhibitors of Bcl-xL and Mcl-1 Anti-apoptotic Proteins

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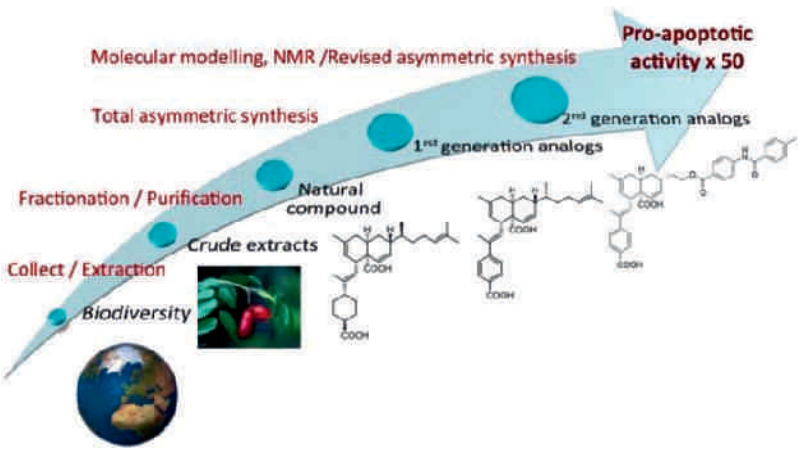
Natural products possess a vast chemical diversity and cover a large chemical space. For these reasons, they are still playing a significant role in the drug discovery and development process. Thus, from the 1940s to date, 75% of the 175 small molecules used in cancer therapy, are either natural products or derivatives of natural products.[1] Screening of plant extracts, marine organisms or microorganisms can provide highly original and functionalized bioactive molecules that are unlikely to be obtained by the screening of synthetic libraries. In fact, chemical complexity is often a criterion of specificity for the target of interest. A few years ago, during a bioassay-guided screening of Malaysian plants extracts, we identified an original triterpenoid, meiogynin A, from the bark of *Meiogyne cylindrocarpa*.[2] This compound acts as an antagonist of the Bcl-xL/Bak and Mcl-1/Bid associations. These proteins are members of the Bcl-2 family, which governs one of the main apoptotic pathways and are often deregulated in many kinds of cancers.[3] We will present the total asymmetric



protecting group free synthesis of meiogynin A,[4] as well as those of more potent analogues[5] for which the synthesis was guided by molecular modelling and protein–ligand NMR experiments. The biological evaluation of all these sesquiterpenoids-type compounds will also be reported.

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LE017 | Aminoglycoside Mechanisms of Action, Resistance and Toxicity—Prospects for Antibiotic Drug Development

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Aminoglycosides are among the most potent antibacterials we have at our disposal, and there is renewed interest in aminoglycosides with a focus on overcoming resistance mechanisms. Aminoglycosides target bacterial protein synthesis by direct interaction with the ribosomal decoding A-site. The phylogenetic variability of rRNA residues provides the basis and the limits for aminoglycoside selectivity. The therapeutic use of aminoglycosides is compromised by significant toxicity, in particular ototoxicity which is irreversible and may result in severe hearing impairment. Few, if any, systemic studies have been done to tune aminoglycoside structures for less ototoxicity. Our previous studies have led to the hypothesis that aminoglycoside ototoxicity is linked to limited target selectivity, i.e., stems from the drug's action on the eukaryotic ribosome.[1,2] Following this mechanism-based hypothesis, we have recently identified and disclosed a set of highly target-selective aminoglycosides.[3,4] The favorable biocompatibility profile combined with the promising antibacterial activity emphasizes the potential of next-generation aminoglycosides in the treatment of infectious diseases without the risk of ototoxicity. Our results validate the potential to modify the neamine core of the aminoglycosides to obtain new generations of this compound class less compromised by adverse side effects.

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LE018 | Visualizing Novel Macrolide Antibiotics Bound to Their Ribosomal Target

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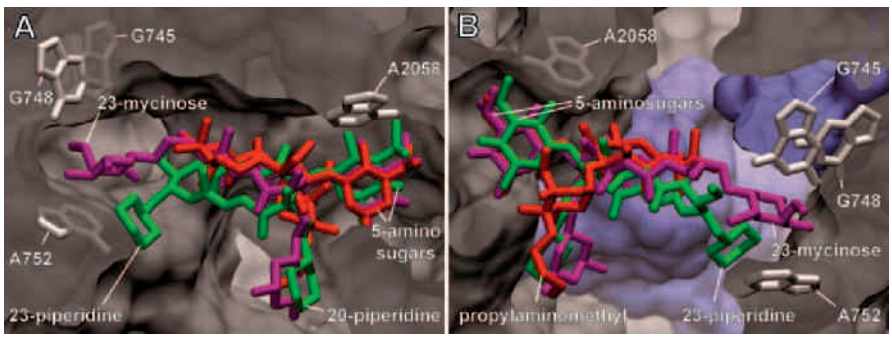
Respiratory tract infections in cattle are commonly associated with the bacterial pathogens *Mannheimia haemolytica* and *Pasteurella multocida*. These infections can generally be successfully treated with several types of veterinary antibiotics including macrolides. Tildipirosin (20,23-dipiperidinyl-mycaminosyl-tylonolide) is a semisynthetic 16-membered ring macrolide derived from the naturally occurring compound tylosin. Tildipirosin (Zuprevo) was recently approved for veterinary use in Europe, the US and Canada. Compared to tylosin and tilmicosin (an earlier tylosin-derivative), tildipirosin is more effective against macrolide-susceptible isolates, and retains activity against some of the resistant strains that have recently emerged.[1–3] Here, the molecular interactions of the macrolides are mapped and visualized at their inhibitory target on the bacterial ribosome.



Chemical footprinting and computer modelling show that tildipirosin, tilmicosin and tylosin all bind and inhibit the well-documented drug site that is centered at 23S rRNA nucleotide A2058 within the large subunit of the bacterial ribosome. There are, however, subtle differences in how the compounds occupy the site. Interactions of the two piperidine components, which are particular to tildipirosin (green), indicate how its mode of action is distinct from tylosin, tilmicosin (magenta) and the 15-membered macrolide tulathromycin (red). The 23-piperidine of tildipirosin contacts specific ribosomal residues on the tunnel wall while its 20-piperidine is oriented into the tunnel lumen and is positioned to interfere with the growing nascent peptide.[4]

We measured the IC_{50} value for tildipirosin at 0.23 ± 0.01 mM in an in vitro assay. The IC_{50} value for tilmicosin was 0.36 ± 0.02 mM, while tylosin and tulathromycin fall between these values. Drug binding is lowered by mutations and methylations of rRNA nucleotides within the target site, and consequently raise MICs.[5] Collectively, the data show how the mode of action of a naturally occurring antimicrobial compound has been improved by derivatization, and provide a basis for further development of macrolide drugs by rational design.

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LE019 | The Ribosome as a Drug Target: Insights from Structural Investigations—Towards Structure-Based Drug Design

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Leishmaniasis, a parasitic disease caused by protozoa of the genus *Leishmania,* affects millions of people worldwide, appearing mainly in tropical and subtropical areas. The disease is transmitted by infected species of sand fly, and can be fatal if untreated. The current state of the art in treating leishmaniasis is based on combined chemotherapy of limited array of drugs.

Aminoglycosides are mostly known as highly potent, broad-spectrum antibiotics that exert their antibacterial activity by selectively targeting the decoding A-site of the bacterial ribosome, leading to aberrant protein synthesis. Recently, aminoglycosides containing a 6'-OH group were highlighted as excellent candidates for the treatment of leishmaniasis. Nevertheless, although some aminoglycosides have already been clinically approved and are currently used worldwide for the treatment of leishmaniasis, the mechanism of which aminoglycosides induce their deleterious effect on *Leishmania* is rather obscure. Based on high conservation of aminoglycosides binding site in bacteria among all kingdoms, it is assumed that the putative binding site of these agents in *Leishmania* is the ribosomal A-site. However, while the recent X-ray crystal structures of the bacterial ribosome in complex with aminoglycosides shed light on the mechanism of aminoglycosides action as antibiotics, no such data is presently available regarding to their putative binding site in *Leishmania*.

Herein, we present the crystal structures of two aminoglycosides bound to their leishmanian binding site: G418, a potent aminoglycoside for the treatment of leishmaniasis at a 2.6 Å resolution, and apramycin, a strong binder for the leishmanian ribosome at 1.4 Å resolution. The observed data provides the first demonstration of aminoglycosides binding to *Leishmania* ribosomes; therefore illuminates the understanding of aminoglycosides mode of action in *Leishmania* at the molecular level. The observed structural data sets ground for the design of new derivatives as potential therapeutic agents against leishmaniasis.

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LE020 | Antagonists of the *Pseudomonas* Quinolone Signal Receptor (PqsR) as Promising Anti-virulence Agents

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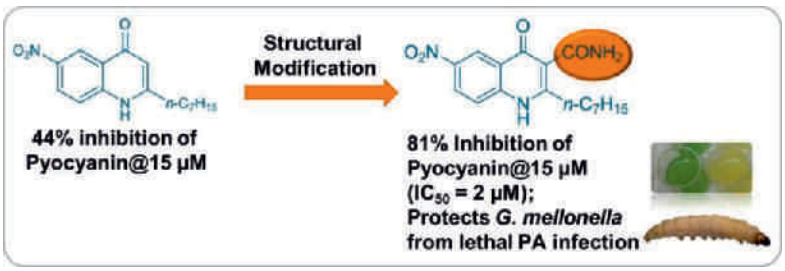
In recent years, an unmet need for novel anti-infective drugs has emerged that emphasizes the importance to discover new strategies to interfere with bacterial pathogenicity.[1] One such approach exploits the ability of microbes to perform concentration-dependent cell-to-cell communication via specific signal molecules also referred to as quorum sensing (QS).[2, 3]

The opportunistic human pathogen *Pseudomonas aeruginosa* possesses the rather unique pqs QS system based on the autoinducer *Pseudomonas* quinolone signal (PQS). This molecule activates the transcription factor PqsR, which leads to the production of virulence

factors like pyocyanin.[4] A disruption of this communication pathway would, in principle, interfere with *P. aeruginosa* pathogenicity without affecting cell viability.[2] Following this concept, we developed potent PqsR antagonists using a ligand-based approach as well as fragment-based strategies.[4,5]

The most promising signal-molecule-derived compound displayed pure antagonism in an *Escherichia coli*-based reporter gene assay in the low nanomolar range. However, an unexpected cell-mediated conversion of this PqsR antagonist to a functional agonist was observed in analogous experiments using *P. aeruginosa* cells.[6] Blockade of an identified "metabolic hot spot" by chemical substitution preserved the desired antagonistic activity. The resulting optimized compound not only caused a significant reduction of pyocyanin production in cellulo, it also demonstrated high in vivo efficacy in nematode (*Caenohabditis elegans*) as well as arthropod (*Galleria mellonella* larvae) infection models.



These results provide a first proof-of-concept for PqsR-targeting anti-virulence agents. Furthermore, discovered fragment-like PqsR antagonists possessing favorable physicochemical properties could pave the way towards drug-like anti-infective agents for the treatment of *P. aeruginosa*-related diseases.[5]

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LE021 | Mechanism-Based Prediction Of Drug-Induced Liver Injury

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Chemical stress is a potentially deleterious state induced by exposure of cells to nonphysiological levels of chemically reactive species (CRS), and it can play a role in off-target adverse drug reactions (ADRs). Whilst we are beginning to understand some of the key events that influence how the liver adapts to exposure to chemicals through the use of animal models, it is not currently known whether humans differ in their ability to mount a defence response and adapt to this stress, with the possibility that this may influence individual susceptibility. If this is the case, this may be through variation in a single biochemical pathway, such as glutathione synthesis, or through the action of one or more proteins that are believed to be the critical early sensors of chemical stress, such as Nrf2. A major challenge in this area is the lack of appropriate and physiologically relevant models of the human liver. The use of primary human hepatocytes is not always straightforward, due to problems associated with supply and quality; moreover, their heterogeneity, partly associated with the intrinsic variability in metabolising enzymes inherent to the population, creates difficulties in experimental reproducibility. Therefore, human hepatocyte model systems are urgently needed. The most widely used human hepatocyte-like cell in compound screening is the HepG2 cell line. However, this is a tumour cell line, and it expresses several-fold lower Phase I and II activities. Clearly there is an unmet need for a relevant and reproducible alternative model to primary hepatocytes or hepatocyte-derived cell lines that emulate human hepatocytes as they exist in the liver. The most likely new source of human hepatocytes for this purpose comes from the embryonic and induced pluripotent stem cell field. This presentation will highlight the key advances in this area and will focus on some critical hurdles that are being addressed by our work in the Mechanism-based Improved Prediction of Drug-Induced Liver Injury (MIP-DILI) IMI programme. The prospect of personalisation in safety assessment, through the great advances in the induced pluripotent stem cell field will also be considered.

LE022 | Strategies to Identify and Manage Risks of Chemically Reactive Drug Metabolites as a Cause of Adverse Drug Reactions

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In recent years, in drug discovery and development novel research strategies have been introduced to include the role of active and reactive metabolites of drug candidates.[1,2] The predictability, and in some cases, the throughput of these research strategies remain a challenge, however. In this presentation, general concepts and recent results of two large multidisciplinary projects (i.e., TI-Pharma ADR and IMI MIP-DILI) will be briefly introduced, and special emphasis will be given on the role of chemically reactive metabolites (CRMs) in this context. Apart from their chemical properties, mechanisms of formation, large-scale production by biocatalysis, identification strategies for adducts between reactive metabolites and proteins as well as their role in selected ADRs will be touched upon. The balance between bio-activation and bio-inactivation processes by, in some cases mediated by genetically polymorphic enzymes, appears crucial when interpreting risks of exposure to chemically reactive metabolites in terms of potential ADRs. Possible strategies for translation from the 'molecule to man' levels will be illustrated as well. Regulatory aspects will be touched upon only briefly.

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LE023 | Being More Certain about Uncertainty in Computational Toxicology Modeling

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Uncertainty arises from many sources in the process of risk assessment. Objective assessment of the sources of uncertainty and quantitation of the effects of this uncertainty on risk assessment conclusions is one of the fundamental challenges for the practice of chemical structure-based toxicological risk assessment. In this presentation, uncertainty will be discussed on two different levels: first at the level of modelling, one must assess the probability of a particular prediction, in this case from a quantitative structure–activity relationship (QSAR) model, to be correct. The second level is on the combination of several pieces of evidence, including QSAR results, structural alerts and structural similarity analysis (read across) into an overall assessment of risk. In both levels we have attempted to adapt mathematical methods from other disciplines in order to better manage uncertainty. In the case of uncertainty in QSAR, we have developed methods for unambiguously assessing whether a new molecule is reliably predicted and in the case of evidence combination, we have investigated methods for evidence combination that clearly identify, highlight and quantify areas of uncertainty.

LE024 | Covalent Modification of Histones by Nevirapine—A Plausible Pathway to Nevirapine-Induced Cancers?

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Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is the most widely used anti-HIV drug in developing countries, both in combination therapy and to prevent mother-to-child transmission of HIV. Whereas NVP is neither mutagenic nor clastogenic in genetic toxicology tests in vitro, it is reported to induce neoplasias in rodents.[1] Moreover, although direct correlation between NVP administration and the onset of human cancer has yet to be reported, epidemiological data suggest an association between the use of NNRTIs and the occurrence of non-AIDS-defining cancers in HIV-positive patients.[2] This implies that these drugs may themselves be carcinogenic, which adds a further concern about their chronic use.

Strong evidence for protein adduct formation by NVP in rodents and humans in vivo was obtained in our research group.[3] By contrast, we found no definite indication of DNA adduct formation by NVP in rodent models in vivo. These data, combined with preliminary reports suggesting that NVP causes epigenetic changes,[4] led us to hypothesize that NVP can covalently modify histones, triggering epigenetic changes that underlie chemical carcinogenesis.

Using proteomic bottom-up strategies, we report herein the first in vitro evidence for the ability of a validated synthetic surrogate of the reactive NVP metabolite, 12-sulfoxy-NVP,[5] to modify multiple lysines in the histone octamer. In vivo studies in rats exposed to NVP are currently underway to establish potential correlations between the occurrence of NVP-promoted histone modifications (and the positions where these modifications occur) and epigenetic changes associated with the onset of cancer.

Acknowledgements:

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LE025 | The Open PHACTS Discovery Platform—Semantic Data Integration for Medicinal Chemists

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Within the last decade, Open Data concepts gained a lot of interest in the area of drug discovery. With the launch of ChEMBL and the availability of PubChem, a large amount of bioactivity data became available to individual medicinal chemists. However, solving complex research questions such as "give me all oxidoreductase inhibitors in human and mouse with an IC_{50} value <100 nM"[1] requires querying across multiple data sources, which deliver their results in different formats, use different standards, and provide their data under different license conditions. Thus, one of the current major challenges is the integration of public data sources in order to allow targeting complex searches via one user interface. The Open PHACTS project, which operates under the framework of the Innovative Medicines Initiative, delivered the publicly available Open PHACTS Discovery Platform, which follows a semantic data integration approach.[2] Via its API, the platform allows to query across multiple domains, such as compounds – targets – pathways – diseases. Furthermore, semantic data integration platforms such as the Open PHACTS Discovery Platform not only enable to query across multiple data domains, they also open hitherto unreached possibilities for in silico model generation and validation. Within this talk, possibilities and limitations when exploiting Open Data will be outlined on basis of concrete use cases for medicinal chemists.

Acknowledgements:

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under Grant Agreement No. 115191 (Open PHACTS), resources of which are composed of financial contribution from the European Union's Seventh Framework Program (FP7/2007–2013) and EFPIA companies' in kind contribution.

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LE026 | European Lead Factory—Game Changing for Innovative Medicines

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The so-called 'patent cliff' has been a much discussed and feared event in the pharmaceutical industry. Numerous blockbuster drugs are coming out of patent in the US and European markets in 2013, and even more by 2015. European pharmaceutical companies alone stand to lose a multi-billion Euro figures if no new products will fill the shrinking drug pipeline. In order to sustain Europe's position in the global health care business new approaches in the pharmaceutical R&D process are being discussed such as Crowd Sourcing and Open Innovation.

The European Lead Factory is a pan-European platform for drug discovery, organized in an Innovative Medicines Initiative (IMI) supported public private partnership (PPP), set to address this issue and to give a major boost to drug discovery in Europe. Comprising a

collection of half a million compounds and a screening center, the European Lead Factory will offer researchers in academia, small and medium-sized enterprises (SMEs) and patient organizations an unprecedented opportunity to advance medical research and develop new drug candidates. The European Lead Factory is a novel platform for innovative drug discovery driven by an international consortium of 30 partners and funded with € 195 Mio. This partnership is the first of its kind and creates unprecedented opportunities for the discovery of new medicines. Academics and SMEs now have access, at no cost to them, to an 'industry-like' discovery platform in order to be able to translate cutting-edge academic research into high-quality drug candidates on a scale and speed that was previously not available to them. This is made possible in part through the access to an exceptional collection of small molecules. 300.000 chemical compounds have been contributed by seven pharmaceutical companies from their corporate chemical collections; an estimated additional 200.000 novel compounds are being jointly developed by academia and SMEs using the integrated knowledge of all consortium partners and through an open innovation and crowd sourcing approach. Researchers form the entire academic community with innovative biology targets as well as chemistry scaffolds are welcome to participate in the EU Lead Factory.

An important part of the European Lead Factory is the European Screening Centre, which assists public contributors of novel targets in the development of assays amenable to the requirements of industrialized screening methodology at no charge to the submitters. The ELF is running a state-of-the-art facility for compound logistics and high throughput screening to handle the 500.000-strong compound collection and provides submitting target owners with Improved Qualified Hit List with relevant screening information with suggestions for progression.

LE027 | Funding Opportunities with the Innovative Medicines Initiative 2

Colm Carroll

Innovative Medicines Initiative, Brussels, Belgium

IMI: The Innovative Medicines Initiative (IMI) was launched in 2008 with the goal of "*significantly improving the efficiency and effectiveness of the drug development process*". A public–private partnership (PPP) between the European Union (EU) and the European pharmaceutical industry (European Federation of Pharmaceutical Industries and Associations; EFPIA), the IMI's €2 billion budget for the period 2008–2013 made it the largest life sciences PPP in the world. This budget has been used to support approximately 60 consortia of academics, small and medium enterprises (SMEs), regulators, patient organisations, and pharmaceutical industry participants addressing issues such as antimicrobial resistance, brain and metabolic disorders, drug discovery, and drug safety.

IMI2: Following the success of the initial IMI, the second phase of the IMI was launched in July 2014 with a new budget of €3.3 billion. The focus of the IMI2 is to develop next-generation vaccines, medicines and treatments. In particular, IMI aims to deliver:

- A 30% better success rate in clinical trials of priority medicines identified by the World Health Organization (WHO)
- Clinical proof of concept in immunological, respiratory, neurological and neurodegenerative diseases in just five years
- New and approved diagnostic markers for four of these diseases and at least two new medicines which could be either new antibiotics or new therapies for Alzheimer's disease.

After a brief introduction to the IMI and some of the ongoing projects, this presentation will focus on opportunities for medicinal chemists under IMI2.

LE028 | PET Imaging: Synthesis of Radiopharmaceuticals and Their Application in Personalized Medicine and Drug Discovery

Peter Scott

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Radiopharmaceuticals labeled with short-lived positron emitting radioisotopes, such as fluorine-18 (half-life=109.77 min) and carbon-11 (half-life=20.38 min), find widespread use in non-invasive positron emission tomography (PET) imaging of biochemical processes in living human subjects. This keynote talk will introduce PET imaging and highlight the main strategies for radiolabeling bioactive molecules with fluorine-18 (including novel approaches to nucleophilic aromatic fluorination reactions, synthesis of [^{18}F]trifluoromethyl groups, radio-click chemistry, and new unconventional approaches to radiochemistry) and carbon-11 (including standard methylation reactions and radiochemistry with [^{11}C]cyanide). Quality control and regulatory oversight associated with radiopharmaceutical production will also be discussed. Finally, imaging applications of radiopharmaceuticals in personalized medicine (e.g., patient stratification and prediction of response to therapy in neurology, cardiology and oncology) and drug discovery (e.g., receptor occupancy studies, patient stratification and monitoring patient response to therapy) will be highlighted.

LE029 | A Rational Approach to the Development of Radioligands and PET Ligands for Drug Development

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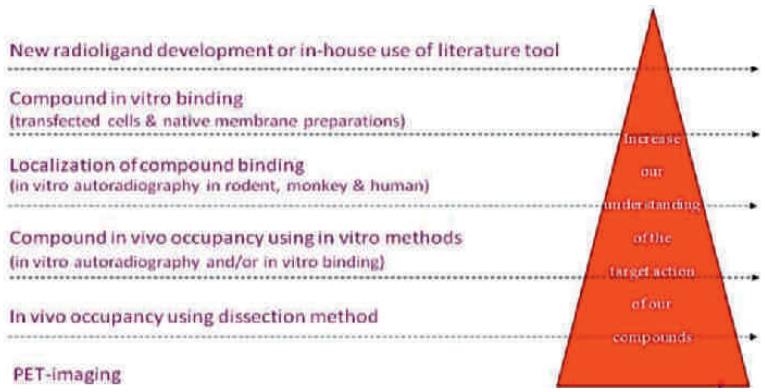
The development of ligands for positron emission tomography (PET) is of critical importance to CNS drug discovery and can be of importance in other therapy areas as well. PET ligands provide for an understanding of the biological action of molecules at the target receptor in the brain by helping to understand target engagement, target response to treatment and receptor occupancy of the drug. The development of novel PET ligands is difficult and we have developed a stepwise process that provides useful data en route to the development of the PET ligand while at the same time improving the chance of a successful delivery. We have applied this process with mixed success, but it has proven to provide better results than less rigorous approaches.



LE030 | Design, Synthesis and Development of Lysophosphatidic Acid Receptor 1 (LPA1) PET Radioligands for Lung Receptor Occupancy Imaging

Samuel Bonacorsi,[1] David Donnelly,[1] Shuyan Du,[1] Adrienne Pena,[1] Joonyoung Kim,[1] Wendy Hayes,[1] Nabeel Nabulsi,[2] Jean-Dominique Gallezot,[2] Yiyun Huang,[2] Richard Carson[2]

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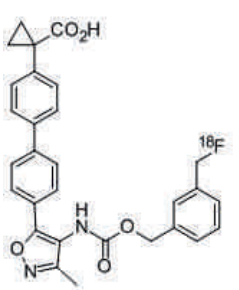
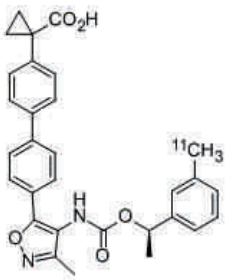
Lysophosphatidic acid (LPA) is a bioactive phospholipid that regulates numerous aspects of cellular function. LPA exerts a wide range of cellular responses and is implicated as a mediator in wound healing and tissue fibrosis. Six isoforms of LPA have been identified, with LPA1 linked to the pathogenesis of lung fibrosis. Antagonism of LPA1 is currently being explored as a target for the treatment of idiopathic pulmonary fibrosis (IPF). It is hypothesized that blocking LPA1 will reduce lung injury by mediating fibroblast recruitment and vascular leakage. A positron emission tomography (PET) radioligand with high affinity for LPA1 would be valuable for establishing target engagement and receptor occupancy relationships for LPA1 antagonists in the human lung. Both [^{18}F]BMT-083133 and [^{11}C]BMT-136088 were designed to be LPA1 PET radioligands, and have low-nanomolar affinity for the LPA1 receptor. Separately, these molecules are functional in ex vivo autoradiography studies and in vivo imaging of LPA1 expression in lung tissue. Here, we describe the discovery and design of these potent LPA1 PET ligands and their preclinical validation using disease state induced models.



[^{18}F]BMT-083133



[^{11}C]BMT-136088

LE031 | Quantitative Imaging of mGlu5 Allosteric Binding Site and its Occupancy by Positron Emission Tomography with the Novel Fluorine-18-Labelled Probe [^{18}F]PSS232

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Background. Positron emission tomography (PET) allows quantification of neuroreceptors and their occupancy by drugs in preclinical and clinical studies. We have recently introduced the fluorine-18-labelled PET probe [^{18}F]PSS232 (see Figure) targeting the allosteric binding site of the metabotropic glutamate receptor 5 (mGluR5, mGlu5).[1] [^{18}F]PSS232 is an analog of the successful [^{11}C]ABP688 with the advantage of a longer half-life of radioactive decay, namely 2 h (^{18}F) versus 20 min (^{11}C). Here, we address its potential to quantify available mGlu5 allosteric binding sites in the rodent brain and make predictions for its use in humans.

Methods. [^{18}F]PSS232 was synthesized as recently described.[1] The IC_{50} value was determined with rat brain homogenate and membranes of hmGlu5-transfected CHO cells (PerkinElmer) with 4 nM [^{3}H]ABP688. The log*D* value was determined in a shake-flask experiment, and free fraction in plasma (f_u) by equilibrium dialysis. Cell-barrier permeation and P-glycoprotein (P-gp) transport were investigated with hMDR1-MDCK cells (Netherlands Cancer Institute), and in vitro metabolism with liver microsomes and an NADH recycling system (BD Biosciences). In vivo experiments were performed with male Wistar rats; PET data was evaluated with PMOD (PMOD Ltd., Switzerland) and MATLAB (MathWorks). Accumulation was expressed as the ratio between the areas under the time–activity curves (0–75 min) of region of interest and cerebellum (reference tissue model) or was evaluated with a two-tissue compartment model with recorded input function (twilite, Swisstrace, Switzerland). Radiometabolites were quantified in plasma and brain homogenates by TLC with a radiodetector.

Results. The in vitro PKPD properties of [^{18}F]PSS232 are ideal for a successful neuroreceptor PET probe. Binding affinities (IC_{50}) to rat and human mGlu5 were 1.3±0.1 nM and 1.1±0.7 nM, respectively, and specific accumulation in autoradiography with rat brain slices agreed with mGlu5 distribution in brain. The log*D* value at pH 7.4 was 2.0±0.1. Free fraction f_u in both rat and human plasma was 0.03. [^{18}F]PSS232 permeation across cells was not affected by P-gp. Incubation of [^{18}F]PSS232 with 0.5 mg rat and human liver microsomes for 60 min reduced the parent tracer to 78% and 94%, respectively. In PET experiments with rats, [^{18}F]PSS232 accumulated in brain regions with high mGlu5 (see Figure). Injection of 1 mg/kg mGlu5 ligand MMPEP during a scan reduced the signal in all brain regions to cerebellum level. Ex vivo biodistribution studies revealed dose-dependent displacement of [^{18}F]PSS232 by MMPEP. Quantitative PET showed high reproducibility; between-subject variability in a test-retest experiment was less than 7.1 % (reference tissue model). Results of the retest scans were lower by ≤7.8 % than those of the test scans (p <0.05), in agreement with a [^{11}C]ABP688 test-retest study.[2] Quantitative PET with an input function allowed kinetic modelling with a two-tissue compartment model. Results were in good agreement with those of the above reference tissue model.

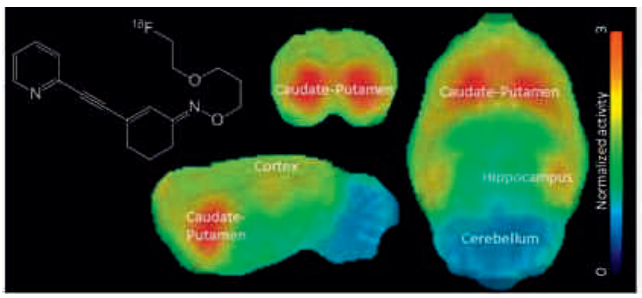

Figure. Structure of [^{18}F]PSS232 and PET scan of a rat brain on an MRI template (25 MBq, 0.2 nmol, 2-90 min)

Conclusion. [^{18}F]PSS232 allows quantification of available mGlu5 allosteric binding sites in rat brain at high reproducibility and low between- and within-subject variability. Quantifications with a simple reference tissue model and a two-tissue compartment model with input function are in good agreement. Defluorination is moderate in rats and is expected to be negligible in humans, based on in vitro metabolism studies.

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LE032 | Experiences with Natural Product Total Synthesis

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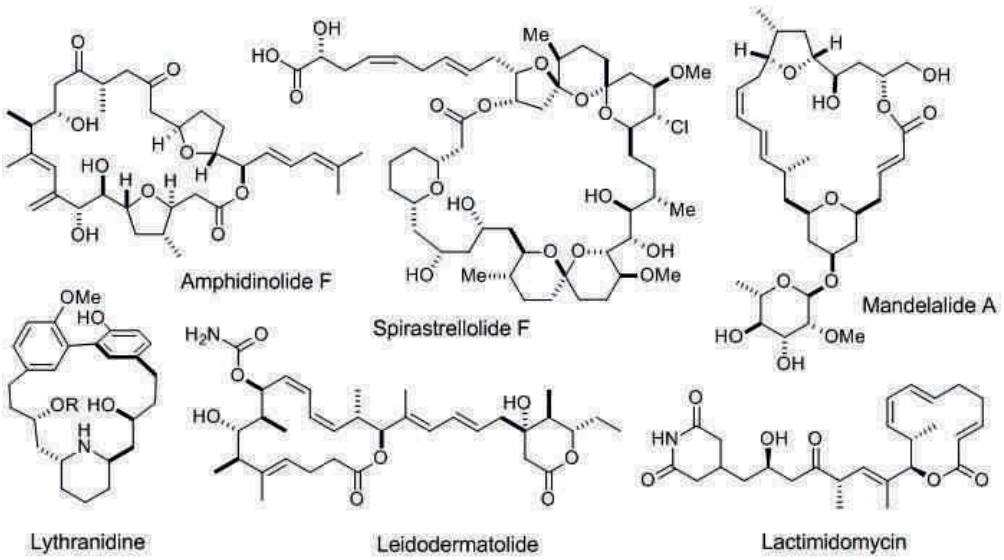
This lecture intends to provide an update on our program concerning the total synthesis and evaluation of complex natural products of biological significance. Targets of current interest include the putative cell-migration inhibitor lactimidomycin,[1,2] the cytotoxic macrolides amphidinolide F and mandelalide,[3,4] the antimitotic agent leiodermatolide,[5] the unusual alkaloid lythranidine,[6] as well as the potent phosphatase inhibitors spirastellolide A and F.[7,8] All syntheses are largely catalysis-based, featuring the scope of the methodology under scrutiny in this laboratory (alkene and alkyne metathesis, platinum and gold catalysis, *trans*-reductions). In some cases our synthesis campaigns led to the revision of the structures and/or bioactivities originally proposed by the isolation teams.



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LE033 | New Synthetic Routes of Nitrogen and Oxygen Heterocycles and Related Compounds

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Oxygen and nitrogen heterocyclic compounds constitute the largest and most varied families of organic compounds, which comprise a great number of classes according to the size, number of heteroatoms and oxidation of the heterocyclic ring. The important industrial and biological applications of these compounds and also some problems associated with their application, such multiple drug resistance to some nitrogen heterocycles and potential carcinogenesis of high doses of oxygen heterocyclic-based antioxidants, led us to develop new synthetic methods for novel derivatives of both families of the referred heterocyclic compounds.

The discovery of rapid, efficient and low-cost methods to construct oxygen and nitrogen has been an important goal in organic chemistry because these ring systems are common structures in natural products and biologically significant molecules. The development of novel stereoselective methodologies and/or the synthesis of enantiopure compounds are a hot topic, since in most of the cases only one of the expected stereoisomers are biologically active.

In the present communication, we will describe our recent results on the development of new synthetic routes: 1) domino multicomponent reactions to generate multiple C–C bonds in a multistep reaction concomitant with the creation of many stereocenters

from simple precursors;[1] 2) synthesis of iminosugars,[2] analogues of pyranoses in which the oxygen atom of the heterocyclic ring is replaced by a nitrogen atom and the anomeric hydroxyl is absent, exemplified by a derivative of a potent inhibitor glycosidases;[3] 3) synthesis of chiral enantiopure 2-*C*-glycosyl-3-nitrochromenes;[4] 4) synthesis of new naturally occurring prenyl-2-styrylchromone derivatives,[5] among others.

Acknowledgements:

Thanks are due to the University of Aveiro, "Fundação para a Ciência e a Tecnologia", European Union, QREN, FEDER and COMPETE for funding the Organic Chemistry Research Unit (project PEst-C/QUI/UI0062/2013) and the Portuguese National NMR network.

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LE034 | Fast and Safe Synthesis of Pharmaceutically Relevant Molecules

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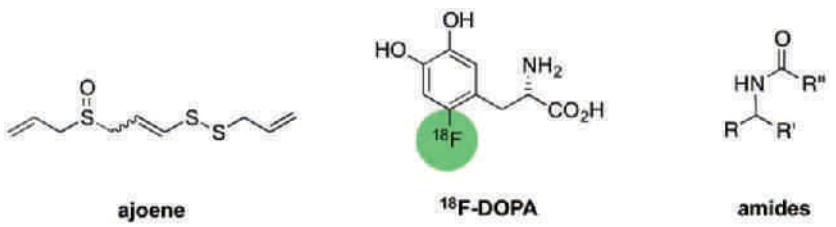
The synthesis and handling of hazardous, dangerous or even only 'unpleasantly smelling' reagents or compounds in the containment of a flow chemistry system has many advantages and is nowadays regularly used on an industrial scale.

The contact between immiscible liquids in a microfluidic system creating segmented flow offers great potential in the study of biphasic reactions in organic chemistry with significant advantages with respect to conventional flask techniques. This can dramatically increase the reaction rate, especially when microwave irradiation, sonication or phase transfer catalysis is combined with segmentation. Metal-catalyzed sequences can also be performed advantageously in biphasic systems.

The potential of handling small volumes in microreactors in a controlled and defined way is used for reactions with hazardous chemicals and also in the development of advanced protocols for the synthesis of radio-pharmaceuticals. [18]-Fluoride radiolabelled compounds were synthesized by different routes using an automated protocol.



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LE035 | Metal-Assisted Synthetic Approaches to Heterocyclic Small Molecules for the Treatment of Cardiovascular Diseases

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Regenerative medicine is understood as one of the most promising therapeutic approaches to alleviate a multitude of diseases. While there is significant progress in the experimental development of this approach related to gene therapeutic intervention often in combination with employing embryonic stem cells, the regulatory implications of such therapies are yet unclear and ethical aspects represent a major obstacle in the further development of such strategies. Hence, small molecules capable of effecting cell differentiation towards particular tissues have received significant attention, as the prospect of employing such agents in regenerative medicine seem highly attractive.

Within the past years, we have been developing several heterocyclic compounds capable of triggering differentiation of certain progenitor cells towards particular tissues otherwise difficult to regenerate. Within a very recent project, we have identified lead compounds incorporating various heterocyclic key motifs capable of efficiently inducing cardiomyogenesis starting from embryonic or progenitor cells, ultimately leading to independently beating heart cells. Considering the fact that cardiac infarction represents one of the major death causes in the developed world, the prospect of tissue regeneration of damaged heart muscle tissue offers a highly innovative perspective of regaining heart function, as this organ in not capable of functional repair on its own.

Synthetic approaches towards the individual target compounds exploited modular strategies based on metal-assisted catalysis, in particular sequential coupling strategies (C-C, C-N, and C-H activation). Up-scaling was conducted employing flow chemistry. Optimization of functional decorations of the particular heterocyclic scaffolds and synthesis up-scaling employing flow chemistry will be discussed in detail.

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LE036 | BETting on Bromodomains: Developing Inhibitors of the Bromodomain–Acetyl-Lysine Interaction

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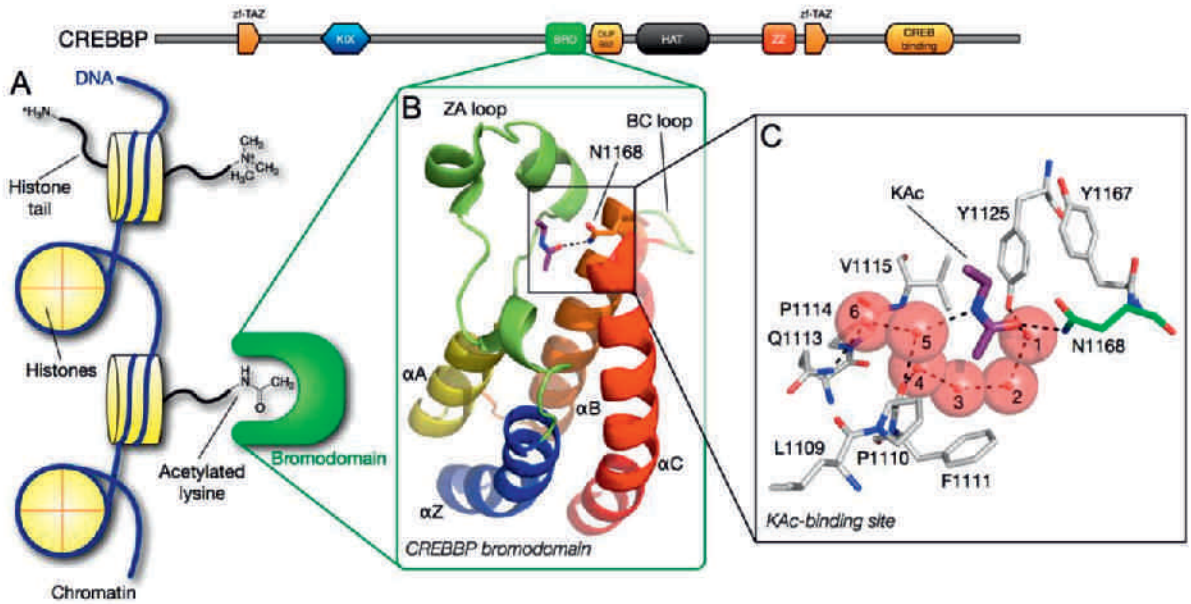
Bromodomains are protein modules that bind to and recognize acetylated lysine residues, and in doing so mediate protein–protein interactions. Protein post-translational modifications (PTMs), including lysine acetylation, occur on histone tails and hence bromodomain-containing proteins (BCPs) are involved in regulating gene expression. There are 61 bromodomains, found within 46 proteins; these modules are emerging important therapeutic targets and the protein–protein interactions they mediate are ligandable.[1]

The bromodomain and extra C-terminal domain (BET) family of BCPs have been the focus of the intense interest and are linked with indications including cancers and inflammation. Here I will describe the fragment-based and structure-guided development of a range of potent BET bromodomain inhibitors. We have evaluated the metabolism of these ligands and will present preliminary pharmacokinetic data on this series of compounds.[2,3] Furthermore, the application of these compounds in a variety of biological settings will be described. More recently, small-molecule ligands for a range of non-BET bromodomains have been reported. I will discuss our work on the fragment-based and structure-guided development of a range of potent CREBBP bromodomain ligands. The tool compounds that we developed have elucidated interesting structural features of the CREBBP bromodomain that contribute to our understanding of ligand SAR.[4]



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LE037 | A First-in-Class Chemical Probe for Family VIII Bromodomains

Dafydd Owen

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Research into the role of epigenetics in disease could be significantly accelerated if cell active chemical probes for such targets were available to the research community, through a collaborative, open innovation model. Pfizer is a member of a public-private partnership led by the Structural Genomics Consortium (SGC) to help identify a suite of high-quality chemical probes for epigenetic targets. This partnership is unique in that it brings the medicinal chemistry expertise within industry together with biological expertise in academia. This collaboration should help drive basic research in an emerging area of important biology, of potential relevance to many diseases.

Mammalian SWI/SNF complexes play a key role in cell differentiation and proliferation, and represent an essential component of the embryonic stem cell. BRG1 (SMARCA4) and BRM (SMARCA2) are the central ATPase components of the multicomponent complexes. BRG1 and BRM are multi-domain proteins that contain a number of DNA and protein interaction modules. These include C-terminal bromodomains. Through collaboration with the Structural Genomics Consortium we have identified a chemical probe that interacts with just three of the Family VIII bromodomains - BRG1, BRM and PB1(5). The discovery, binding mode and phenotype derived from the use of PFI-3 will be discussed.

LE038 | Epigenetic Drug Discovery: Small-Molecule Inhibitors of Class IV Bromodomain Proteins

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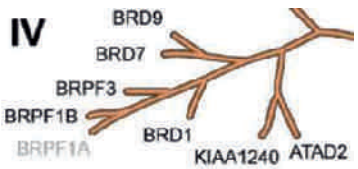
The human BRPF (bromodomain and PHD finger containing) family of histone acyl-lysine reader proteins, BRPF-1, -2, -3, are important regulators of epigenetic signalling. These proteins recognise specific acyl lysine residues on histones, leading to changes in chromatin structure, multi-protein complex formation and transcriptional regulation. This drug target is relatively early with respect to target validation, although there is an emerging understanding of its potential role in acute myeloid leukemia (AML).[1,2] The activation of the BRPF1/HOX pathway through MOZ histone acyl transfer is critical for MOZ-TIF2 to induce AML.



In collaboration with the Structural Genomics Consortium (University of Oxford, UK), we have identified potent and selective small-molecule inhibitors of BRPF1 through optimization of a fragment-derived screening hit. In addition, small-molecule inhibitors of other class IV bromodomain proteins have been investigated by opportunistic screening of compounds prepared by this programme. Ultimately, these inhibitors may represent potential starting points for a drug discovery program as a new approach to the treatment of AML and possibly other cancers.

Acknowledgements:

SGC: Oleg Fedorov, Octovia Monteiro, Sarah Martin, Cynthia Tallant Blanco, Chela Nunez Alonso, Bethany Jose, Susanne Muller-Knapp, Paul Brennan and Stefan Knapp. **UCL**: Niall Igoe and Elliott Bayle. In addition, we are grateful to CRUK-CRT and CRUK Manchester Institute.

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LE039 | Interrogating the Bromodomain Family through Chemical Biology

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The bromodomain family of acetyl-lysine binding proteins is extremely well suited to a chemical biology approach. While the bromodomain–ligand interactions are formally protein–protein interactions, the binding sites are in many cases quite druggable. In addition, sequence analysis across the family of binding sites reveals a perfect blend of sequence similarity to enable target hopping as well as sequence differences that allow the development of highly selective probe molecules. Our strategy is to make potent, selective, and cell-active probes against the entire bromodomain family, and then to use these probes to broadly interrogate biology in different contexts. We will discuss the status of our platform activities, as well as give examples of phenotypic responses that result from selective engagement of particular bromodomains.

LE040 | Development of New Anti-infective Agents and a Novel Delivery Strategy

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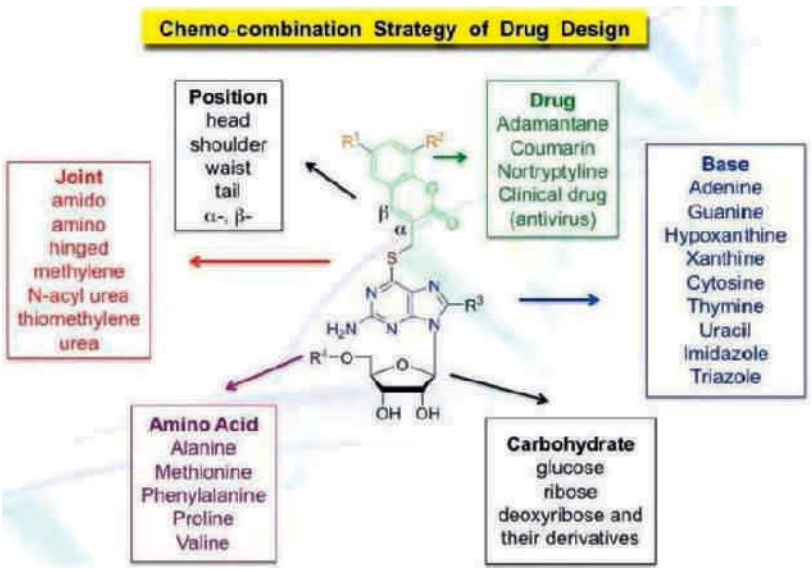
2) Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

After three years of research on the SILVER project of the 7th Framework Program funded by European Commission, our lab has successfully established 12 compound libraries. More than 660 new conjugated compounds containing nucleoside, heterocycle, coumarin, and sulfonate moieties as shown below were designed and synthesized, of which the anti-vial activities were explored by our SILVER Consortium partners. Results from Leuven University (Belgium) indicate that more than 10 compounds possessed potency and identified as hits against chikungunya and hepatitis C viruses. It is the aim of the SILVER project to develop new drugs for emerging, re-emerging, the relatively neglected diseases caused by RNA viruses.

Moreover, single-stranded DNA of mixed dA, dT, dG, and dC was used for the first time to wrap around functionalized single-walled carbon nanotubes. Their external was covalently attached with multiple triazole–(ethylene glycol)–dA ligands. This approach of hybridization involved the formation of hydrogen bonds between dT of single-stranded DNA and dA of functionalized carbon nanotubes. These functionalized nanotubes can be applied as a new type of vehicle for delivery of anti-infective drugs.



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LE041 | Synthesis of Anti-infective Cyclic Natural Products and Natural Product-Like Compounds

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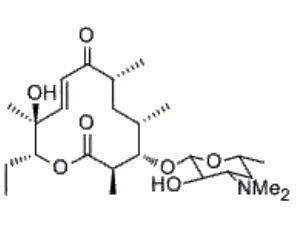
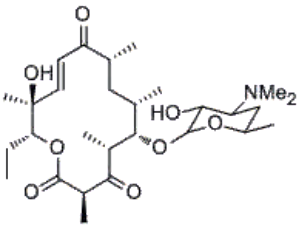
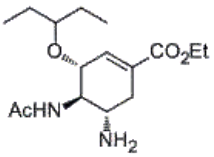
Macrolide antibiotics are categorized as an important class of compounds exhibiting clinically interesting biological activities. Structurally, they have large lactone rings as aglycones attached to one or more sugars. Erythromycin has been the most exposed and popular macrolide antibiotics probably because of its superior anti-infective activities. In contrast, pikromycin has been relatively neglected even though it was the first known macrolide antibiotic isolated in 1950. A similar group of macrolide antibiotics is also produced by *Streptomyces venezuelae*, that is, the methymycin family that contains 12-membered macrolactones as aglycones. We have been involved in the total synthesis of pikromycin and methymycin families of natural macrolide antibiotics. Enhancement of biological activity is desirable to apply methymycin and pikromycin families of antibiotics to clinical use. Inspired by ixabepilone as an example of the analogue-based drug design, we have been intrigued by the possibility of enhancing the metabolic stability that would lead to the improvement of the level of anti-infective activities. We have successfully achieved the synthesis of pikromycin and methymycin families of macrolides and the lactam analogs of these macrolide antibiotics.



methymycin



pikromycin



Oseltamivir (Tamiflu)

Neuraminidase has been a popular target for the drug research because it plays an important role in cleaving sialic acid residues. Oseltamivir (Tamiflu) is the most efficient anti-influenza drug ever developed and it was designed to mimic the transition state according to the proposed mechanism of the action of neuraminidase. We were interested in the synthesis of oseltamivir. Our synthetic strategy was based on the utilization of *cis*-2,3-bis(hydroxymethyl)aziridine as a key starting material and the cyclization by ring-closing metathesis. We have also extended this synthetic strategy to prepare some natural product-like analogs including carba-sugar derivatives hoping to contribute to the development of sugar-based glycosidase inhibitors.

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LE042 | Novel Boron-Containing Anti-infective Agents

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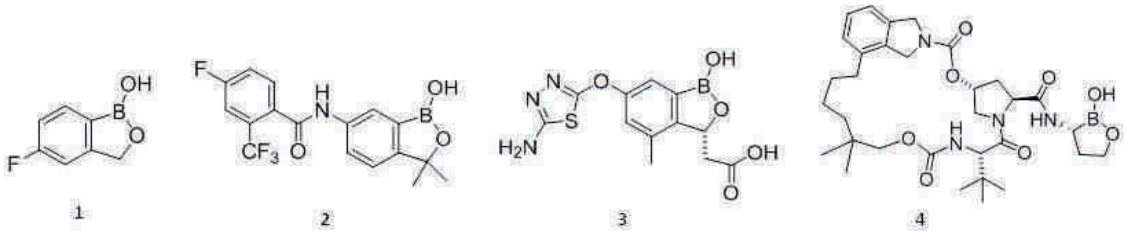
Boron has unique and valuable properties as a building block in the design and creation of new, small-molecule pharmaceuticals. Due to its vacant p-orbital, boron-containing compounds are capable of forming high affinity, selective and reversible complexes with sugars and amino acids that contain a Lewis base donor. This mechanism provides an opportunity for these compounds to interact with target proteins and to generate drug molecules of high potency and high ligand efficiency. Over the last decade, pioneering research has demonstrated boron's potential in therapeutic areas including cancer, infectious diseases and inflammation.

Anacor Pharmaceuticals is focused on discovering new therapeutic agents by inclusion of boron into small-molecule templates. We have created a library of boron-containing compounds that has proven to be a rich source for both biochemical and phenotypic screening. In addition, we have discovered novel lead compounds by incorporating small oxaborole fragments into existing bioactive scaffolds. Utilizing these two approaches to generate promising lead compounds, we initiated R&D programs seeking to discover new anti-infective drugs that show improved properties to existing agents. Focusing on microbial and viral infections, we used well-established medicinal chemistry practices, including structure-guided lead optimization, to discover compounds **1**, **2**, **3** and **4**. These novel anti-infective compounds have now progressed into preclinical and clinical studies for the potential treatment of onychomycosis, human African trypanosomiasis, Gram-negative bacterial infection and HCV infection, respectively.[1–4] This presentation will review our experience researching and developing boron-containing small molecules as new anti-infective agents.



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LE043 | Lectins as Targets in Antiadhesion Therapy—Example of DC-SIGN and FIMH Antagonists

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Present drug discovery of novel anti-infective agents is still predominantly governed by more than a century old Paul Ehrlich's paradigm of the "magic bullet" with the clear aim of direct bactericidal or bacteriostatic activity. The alternative strategy to fight bacterial or viral infections is the so-called anti-adhesion therapy.[1] If one focuses on bacteria only, their ability to infect host tissues is governed by its adhesion to a specific tissue, and the inhibition of this process is considered an efficient way to stop infection and biofilm formation.[2] Unfortunately, no antibacterial anti-adhesion agent is currently registered and although the picture is somehow more optimistic in the antiviral field, only a handful of these drugs reached the market.

Bacterial and viral surface lectins that adhere to glycosylated host proteins or human lectins that bind pathogenic glycosylated surface proteins are the most common adhesion molecules and virulence factors that guide viruses and bacteria's adhesion ability and tissue selectivity. DC-SIGN or "Dendritic Cell-Specific ICAM-3 Grabbing Nonintegrin" is a C-type lectin on dendritic cells involved in the pathogen binding, recognition and clearance in the early stages of the infectious process. DC-SIGN specifically recognizes complex mannose- and fucose-based glycans on the microorganism and endogenous cell surface. Certain pathogens bind to DC-SIGN, but escape the normal antigen processing and presentation on dendritic cells. Instead, these pathogens use DC-SIGN as a Trojan horse for host cell invasion, which is the primary mechanism of infection with HIV.[3] DC-SIGN antagonists are potential antiviral and antibacterial agents, but no such agent has yet been discovered. DC-SIGN antagonists could prevent HIV infection in the very early stage of infection, which is a new concept for prevention of HIV infection. Our group was involved in the design, synthesis and evaluation of D-mannose and L-fucose based glycoconjugates that bind to DC-SIGN with affinity up to 3 orders of magnitude greater than the native monosaccharide. Furthermore, they were proven to antagonize HIV gp120 binding to primary dendritic cells pointing to their therapeutic potential in the prophylaxis of HIV-1 infection.[4]

Uropathogenic *Escherichia coli* (UPEC) provokes the vast majority of uncomplicated urinary tract infections as it invades urinary tract by adhering via fimbrial lectin FimH.[5] It is a D-mannose selective adhesin that allows UPEC binding to the luminal surface of urothelial cells. Accordingly, the inhibition of FimH by a small-molecule FimH antagonist might be used to treat UPEC. We have recently reported that α-D-mannopyranosides bearing diaryl substituted 1,3-diaminopropanol or glycerol moieties act as potent FimH antagonists in vitro, as determined by a competitive binding assay on isolated FimH lectin.[6] Most of the assayed compounds display nanomolar FimH antagonistic activity. Based on promising results of the first series of compounds, we have designed and synthesized a new series of asymmetrically disubstituted glyceryl α-D-mannopyranosides with improved physicochemical properties. Furthermore, compounds were found to be non-cytotoxic on HepG2 cells in concentrations up to 10 μM pointing to their selective toxicity, which is one of key features of potential therapeutics for the treatment of urinary tract infections. All these compounds follow the same archetype of glycomimetic design, where the "core monosaccharide" is utilized to selectively anchor the ligand in the lectin binding site, while the aglycon moiety attached to the anomeric center boosts the binding affinity.[7] This general approach might be recognized as an attractive route towards new lectin antagonists as novel anti-infective agents.

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LE044 | Therapeutic Opportunities Offered by the Excessive Lactate Production in Cancer

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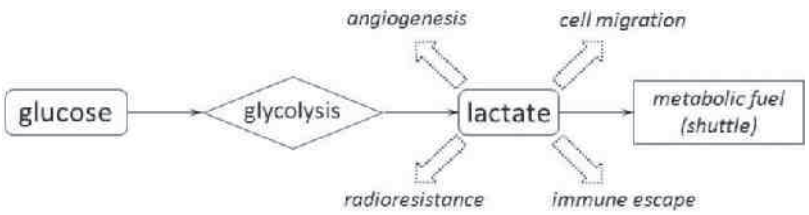
The majority of cancers of various tissue origin display wide portions suffering from insufficient respiration, due to permanent or transient hypoxia, which occurs during tumor development. This condition leads to the development of a glycolytic phenotype, where a compensatory lactate production takes place, in order to provide the cancer cells with sufficient amounts of energy and anabolites. Lactate is not just a waste product of the glycolytic process; instead, it plays a key role in the progression of cancer, since it promotes angiogenesis, cell migration, immune escape and radioresistance. Moreover, lactate can still constitute a metabolic fuel for oxidative tumor cells or vascular endothelial cells, and it can establish a symbiotic cell–cell shuttling system with stromal cells. Therefore, these peculiar roles of lactate in invasive tumors constitutes a high-priority target for future anticancer therapeutics.[1]



Therapeutic interventions aimed at reducing lactate production in cancer tissues may consist of: a) reduction of glucose uptake (calorie-restricted ketogenic diet, physical exercise, inhibitors of glucose transporters); b) inhibition of enzymes involved in key steps of glycolysis (inhibitors of hexokinase, phosphofructokinase, lactate dehydrogenase); c) block of the cellular trafficking of lactate (inhibitors of monocarboxylate transporters); d) enhancement of the mitochondrial oxidative metabolism (hyperbaric oxygen therapy, removal of inhibition of the Krebs cycle, for example, by using inhibitors of pyruvate dehydrogenase kinase).[2]

We have developed compounds that exert an antiproliferative action on cancer cells by specific interventions on cancer metabolism, such as, inhibition of lactate dehydrogenase (LDH) activity,[3,4] or reduction of glucose uptake through specific transmembrane transporters (GLUTs).[5] Furthermore, some of the LDH inhibitors demonstrated a remarkable synergism with gemcitabine against pancreatic cancer cells in hypoxia,[6] and an improved activation of the redox-sensitive anticancer prodrug EO9 by means of an induced increase of the $NADH/NAD^+$ cell ratio.[7]

It is important to note that the development of agents that target lactate production, trafficking, and metabolism (by these or other methods) hold promise for treating nearly all invasive cancers, provided they present an appropriate therapeutic window.

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LE045 | Novel Cancer Treatment Targeting Vps34

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Autophagy is a catabolic process that degrades organelles and proteins in the lysosomal machinery, thereby ensuring cell survival during starvation and stress. Autophagy can be induced by amino acid and glucose withdrawal, but also during drug treatment, as shown in several studies of inhibitors of HDAC, BRAF, proteasome, mTOR and tyrosine kinases. Therefore autophagy is postulated to be a key resistance mechanism in cancer treatment.

The lipid kinase activity of phosphatidylinositol 3-kinase class III, Vps34, is necessary for the nucleation of autophagic vesicles and the formation of autophagosomes.

In the development of autophagy inhibitors, a fragment-based drug development approach has identified a series of potent and selective inhibitors of Vps34 with high ligand efficiency (LE>0.4), good drug-like and preclinical properties. The inhibitors show potencies in the low nanomolar to picomolar range against Vps34 and a 100-fold or more selectivity towards the class I isoforms as well as mTOR. The series is devoid of kinase activity in a broad kinase screen and has excellent solubility, permeability and metabolic stability. Representatives of the series have been tested in pharmacokinetic studies in mice and demonstrated good bioavailability and half-life.

We have developed a high-content screening autophagy assay using a stably transfected GFP-LC3 cell line and shown that our Vps34 inhibitors demonstrate a profound decrease in autophagic flux. We have also studied the autophagy dependence in a triple negative breast cancer cell line, MDA-MB-231, during drug treatment and metabolic stress in relation to Vps34 expression, endogenous LC3-II and other autophagy markers.

We have demonstrated that Vps34 inhibitors potentiate the effect of cytotoxic drugs and targeted therapy in triple negative breast cancer cells. We are currently studying the effect of autophagy inhibition on glucose uptake. This study will shed light on the utility of Vps34 inhibitors in cancer treatment.

LE046 | Exploiting Tumor Metabolic Vulnerabilities with Novel Oxidative Phosphorylation (OxPhos) Inhibitors

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Inhibition of mitochondria complex I in tumors that are metabolically dependent on oxidative phosphorylation (OxPhos) for their survival offers unique synthetic lethal opportunities. Examples of dependent contexts are acute myeloid leukemia (AML) and diffuse large B-cell lymphoma (DLBCL), where OxPhos is highly active, and subpopulations of glioblastoma and neuroblastoma, which possess genetic alterations that render them glycolysis deficient. In addition, several lines of evidence indicate that after treatment with chemo or targeted therapy, residual tumor cells become reliant on OxPhos for their continued survival. In each of these cellular states, excessive dependence on OxPhos renders tumor cells vulnerable to therapeutic targeting strategies that exploit this addiction.

We have generated a series of novel, highly potent complex I inhibitors, which in vitro inhibit isolated complex I with IC_{50} values between 1–10 nM. Lead compounds specifically induce apoptosis with EC_{50} values between 1–10 nM in OxPhos-dependent AML and DLBCL cell lines and in glycolysis-deficient cancer cell lines.

The initial focus of the medicinal chemistry team was on improving the pharmacokinetic profile of these initial leads, and resulted in the identification of orally bioavailable compounds with excellent pharmacokinetics properties in preclinical species, making them appropriate tools for proof-of-concept studies in vivo. In agreement with data in cell culture, we have shown that daily oral treatment with our OxPhos inhibitors is well tolerated, induces strong regression of glycolysis-deficient gliobastoma and DLBCL xenografts models, and dramatically increases mice survival in an orthotopic AML xenograft model.

Following the compelling results obtained with this first set of in vivo tool compounds, extensive effort focused on the optimization of the physicochemical properties and overall drug-like profile of our OxPhos inhibitors, resulting in the identification of a preclinical candidate. Aspects of the medicinal chemistry strategy leading to selection of the current lead molecule will be discussed, along with the strong antitumor efficacy observed in the relevant xenograft models.

LE047 | Discovery and Characterization of A-366, A Novel, Potent and Highly Selective Inhibitor of Histone Methyltransferase G9a

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The role of epigenetic alterations in cancer development and maintenance is currently an area of intense research. It is hoped that a deeper understanding of epigenetic dysregulation mechanisms will lead to the next generation of oncology therapeutics. The histone methyltransferase (HMT) class of enzymatic writers of epigenetic marks is one of the classes of epigenetic modulators that may provide targets of therapeutic value. HMTs catalyze the methylation of histone lysines and arginines, a process that can result in either activation or repression of transcription. G9a (EHMT2) is a member of the HMT family that has been implicated in a variety of cancers. In order to further understand the role of G9a enzymatic activity in cancer and evaluate the potential of G9a inhibitors as therapeutic agent we developed a highly selective and potent inhibitor, A-366 that is suitable for in vivo evaluation in long term tumor growth inhibition xenograft models. A-366, discovered after optimization of a screening hit, is a peptide competitive inhibitor of G9a and the closely related GLP (EHMT1) with greater that 1000-fold selectivity over 21 other methyltransferases. The mode of binding was confirmed via an X-ray co-crystal structure of A-366 with G9a. Significantly, A-366 appears to be a more selective probe molecule than known G9A inhibitors as it displays lower cytotoxicity toward solid tumor cell lines, despite roughly equivalent cellular inhibitory activity on histone methylation. A-366 was found to have a cytostatic role in various leukemia cell lines and was tested in vivo in a leukemia model (MV4;11 flank xenograft) resulting in growth inhibition consistent with reduction in H3K9me2 levels. In summary, A-366 is a highly selective G9a/GLP inhibitor that can shed further light on the role of G9a in cancer progression and maintenance.

LE048 | Integration of New Chemical Diversity and Design Approaches in Lead Generation

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The genomic revolution has provided an explosion of “potential” drug targets, however the translation of these targets into medicines that impact and improve the lives of patients has not proceeded at the pace that was anticipated. Many factors have contributed to this so-called “innovation gap” in drug discovery and, in general, these factors can be categorized as target-related or compound-related. Target-related factors concern the innate relationship of the target to human disease and are confirmed, but not altered by the drug discovery process. As medicinal chemists, however, compound-related factors are directly under our control.

Because there is incomplete overlap between “drug-like chemical space” and “disease-relevant target space”, we are often faced with a choice between targets that we know how to drug and those that we know will work in the clinic. Existing chemical libraries have generally been constructed to emphasize traditional concepts of drug-likeness, even though many highly desirable targets/target families have been refractory to this sampling of chemical diversity. Although the set of hypothetical organic chemical structures is essentially limitless, resource constraints have steered researchers toward techniques that will generate the most compounds in the shortest time, rather than broadly interrogating “chemical space”. This talk will survey recent approaches to broaden our access to chemical space, including in silico approaches, fragment-based approaches, automated synthetic methods, and open innovation. These modern lead generation strategies are expanding our concepts of drug-likeness through the exploration of more complex and topologically diverse structures, and have the potential to open new opportunities for therapeutic innovation.

LE049 | Automated Design of Bispecific Inhibitors

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A drug’s activity profile, across multiple proteins in protein network, determines its clinical efficacy and safety. Methods to rationally design drugs a priori against polypharmacologic profiles of multiple proteins would have immense value in drug discovery. Recently we presented a new approach for the discovery of ligands, by the automated, de novo design of compounds against multitarget (polypharmacology) profiles. In this presentation, we shall outline the rationale for developing small-molecule ‘bispecific’ ligands to selectively modulate distinct pathways that are hypothesized to enhance efficacy. We shall demonstrate proof-of-concept examples of the automated design of first-in-class bispecific inhibitors, for novel combinations of targets. In order to design bispecific ligands, we have extend our technology platform to integrate both machine learning and structural bioinformatics into an evolutionary drug design algorithm.

LE050 | Starting Small, Thinking Big: The Application of Fragment-Based Drug Discovery to Lead Generation

Steven Woodhead

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Fragment-based drug discovery (FBDD) has established itself as a viable and productive approach to lead generation and the optimization of novel small-molecule drugs. Fragments are generally characterized by their low molecular weight and low structural complexity, which permits weak, but highly efficient binding to biologically relevant macromolecules. By virtue of their weak binding affinity, fragment screening is typically executed using sensitive biophysical techniques in parallel with high concentration biochemical methods. Once hits have been fully characterized, efficient optimization is ideally driven by iterative structure-guided design, supported by X-ray crystallography.

The successful application of fragment methods is dependent on a number of factors, including well characterized fragment libraries, an integrated biophysical platform of screening and characterization techniques, and strong cross-functional collaboration. This presentation will describe Takeda's approach to FBDD, including library design, the application of biophysical techniques, and how FBDD has been successfully employed as part of an integrated approach to lead generation. Examples of efficiency-driven optimization of fragment hits to high-quality leads will also be presented.

LE051 | Discovery of the First and Only Small-Molecule IL-17 Blockers

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IL-17 is a clinically validated target implicated in multiple inflammatory and autoimmune diseases. Discovery of small-molecule antagonists of protein–protein interactions, like IL-17 and its receptor, has been limited due to technological hurdles. Here, we present the discovery of the first small-molecule (MW<500 Da) IL-17 antagonists. The discovery was made using a DNA-encoded chemical library and a recently developed homogenous screening assay. The assay employs a unique principle of trapping small-molecule binders together with the protein target in miniscule droplets. The screen resulted in more than 800 primary hits, constituting three hit series with clear instant structure–activity relationships, which may accelerate the hit-to-lead transition and the following lead optimization process. Hits were identified as having nanomolar potencies, MW 500 Da, and cLog*P* values of approximately 2.5, thus providing promising starting points for developing orally bioavailable therapeutics.

LE052 | Discovery and Development of Surotomycin for the Treatment of *Clostridium difficile*

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Surotomycin is a novel, orally administered, cyclic lipopeptide antibiotic currently in development for the treatment of *Clostridium difficile*-associated diarrhea (CDAD). Surotomycin has potent activity against Gram-positive organisms, including *C. difficile* and vancomycin-resistant enterococci (VRE). Surotomycin is rapidly bactericidal, and at 16X the MIC produced >3-log reduction of *C. difficile* in a time-kill assay. It displays a prolonged, dose-dependent postantibiotic effect (>6 hours at 80X MIC, a concentration easily achieved in vivo). Spontaneous resistance at 8X the MIC was below the limit of detection in *C. difficile*, and below the limit of detection for enterococci at 16X MIC. Under selective pressure by serial passage against *C. difficile*, the susceptibility of the bacteria changed no more than twofold during 15 days of serial passages. No cross-resistance is seen with other agents used in the treatment of *C. difficile*. In the hamster model of CDAD, surotomycin demonstrated potent dose- and time-dependent efficacy in resolving initial disease onset, even at very low doses.

Surotomycin has limited activity against Gram-negative GI facultative and strict anaerobes including bacteroides. As a result, while both surotomycin and vancomycin alter the gastrointestinal flora of treated animals and patients, the patterns of alteration are different and may influence clinical outcomes. Surotomycin is also more rapidly bactericidal in vitro than vancomycin, and lipopeptides retain activity against stationary phase bacteria; these features could also influence clinical outcomes. In Phase 2 trials, surotomycin dosed at 125 or 250 mg/day for 10 days produced equivalent end-of-therapy cure rates to vancomycin (92.4%, 86.6%, and 89.4%, respectively). However, surotomycin was superior with regards to recurrence rates (27.9%, 17.2%, 35.6%), resulting in higher rates of sustained cure (66.7%, 70.1%, 56.1%). Ongoing Phase 3 studies will determine whether these findings are reproducible in a broader patient population.

LE053 | MBX-500: A Hybrid Antibiotic with In Vitro and In Vivo Efficacy Against Toxigenic *Clostridium difficile*

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Clostridium difficile infection (CDI) causes moderate to severe disease resulting in diarrhea and pseudomembranous colitis. CDI is difficult to treat due to production of inflammation-inducing toxins, resistance development, and the high probability of recurrence. Very few antibiotics are approved for the treatment of CDI, and the pipeline for new therapeutic agents is small. MBX-500 is a hybrid antibacterial, composed of an anilinouracil DNA polymerase inhibitor linked to a fluoroquinolone DNA gyrase/topoisomerase inhibitor, with potential as a new therapeutic for CDI treatment. Since MBX-500 inhibits three bacterial targets, it is minimally susceptible to resistance development. We have examined the in vitro and in vivo efficacy of MBX-500 against the Gram-positive anaerobe, *C. difficile*. MBX-500 displays good potency across nearly 50 isolates, including those of the fluoroquinolone-resistant, toxin-overproducing NAP1/027 ribotype, and performing as well as the comparator antibiotics vancomycin and metronidazole. Furthermore, MBX-500 was found to be a relatively narrow-spectrum agent, displaying poor antibacterial activity against many natural gut anaerobes. MBX-500 was active in both acute and recurrent infections in a toxigenic hamster model of CDI, exhibiting full protection against acute infections and prevention of recurrence in 70% of the animals. Hamsters treated with MBX-500 displayed significantly greater weight gain than did those treated with vancomycin. MBX-500 was also efficacious in a murine model of CDI, again demonstrating a fully protective effect and permitting near-normal weight gain in the treated animals. Finally, in a new piglet model of CDI, which mimics many of the key characteristics observed in human CDI, MBX 500 was fully efficacious, improving clinical signs, reducing lesion severity, and reducing toxin and cytokine levels when compared to untreated controls. These selective anti-CDI features support the further development of MBX 500 for the treatment of CDI.

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LE054 | Targeting Spore Germination to Prevent *Clostridium difficile* Infections

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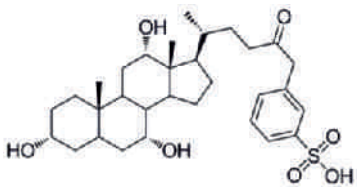
Clostridium difficile infection (CDI) is the major identifiable cause of antibiotic-associated diarrhea. In the US, there are over 500,000 cases of CDI annually, with a mortality rate of up to 2.5 % and costs up to $3.2 billion.[1] CDI is treated with vancomycin or metronidazole, but treatment failure and relapse are common. The infective form of *C. difficile* is the spore, a dormant and resistant structure that forms in response to nutrient deprivation.[2] *C. difficile* spores do not cause disease but germinate into toxin-producing cells in the microbiota-depleted gut of patients. Because *C. difficile* spore germination is required for symptomatic infection, anti-germination approaches could lead to the prevention of CDI.[3]

We recently tested different bile salts for efficacy in activating and inhibiting *C. difficile* spore germination.[4] We found that CamSA (see Figure), an *m*-aminobenzene sulfonic acid bile salt derivative, inhibits spore germination both in vitro and in vivo. More importantly, we found that a single 50 mg/kg dose of CamSA, was sufficient to prevent CDI in mice without any observable toxicity.[5] We also showed that CamSA partially protects hamsters from CDI relapse. We further characterized CamSa's in vitro stability, distribution, and cytotoxicity. We reported that CamSA is stable to simulated gastrointestinal (GI) environments, but will be slowly degraded by members of the natural microbiota found in a healthy gut. Our data also suggested that CamSA will not be systemically available, but instead will be localized to the GI tract. CamSA shows no toxic effects towards vegetative bacteria, epithelial cells or macrophages.[6] Several experiments support a mechanism whereby the anti-germination effect of CamSA is responsible for preventing CDI signs. Lower CamSA doses resulted in delayed CDI onset and less severe signs of disease.[5] By varying the timing of CamSA dosage, we estimated that *C. difficile* spores germinated and established infection less than 10 hours after ingestion. We also showed that ingested *C. difficile* spores rapidly transited through the GI tract and accumulated in the colon and cecum of CamSA-treated mice. From there, *C. difficile* spores were slowly shed over a 96-hour period and were quantitatively recovered from feces and intestinal content.[6]



To our knowledge, this is the first report of using molecular probes to both prevent CDI and obtain disease progression information for *C. difficile* infection. This approach represents a new paradigm in CDI treatment. Instead of compromising further the microbiota of

CDI patients with strong antibiotics, anti-germination therapy could serve as a microbiota surrogate to curtail *C. difficile* colonization of antibiotic-treated patients.

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LE055 | Cadazolid: A New Antibiotic in Development for the Treatment of *Clostridium difficile*-Associated Diarrhea (CDAD)

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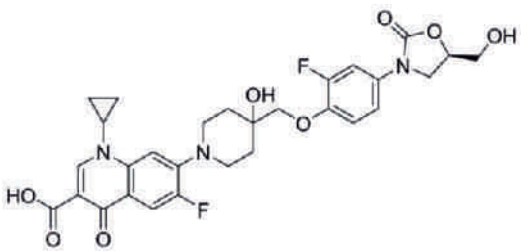
Clostridium difficile-associated diarrhea (CDAD) is a major healthcare problem and a leading cause of morbidity and mortality in elderly hospitalized patients. CDAD results from overgrowth of toxin-producing strains in the colon, typically following disturbances of the normal protective enteric microflora caused by broad-spectrum antibiotic therapy. The incidence and severity of CDAD has increased in the past decade, and new hypervirulent strains of *C. difficile* have been observed. Current antibiotic therapies for CDAD include vancomycin and metronidazole, which have limited treatment success in severe disease and disease recurrence rates up to 30%. Fidaxomicin, a recently approved antibiotic, had similar cure rates as vancomycin and showed overall less recurrence, except for infections involving the hypervirulent NAP1/BI/027 strain. Thus there remains a need for new drugs with improved efficacy.



Cadazolid

Cadazolid is a new oxazolidinone antibiotic with a quinolone moiety showing potent activity against *C. difficile* and other Gram-positive bacteria, overcoming all major resistance types and exhibiting a very low propensity for resistance development.[1] It acts primarily as an inhibitor of bacterial protein synthesis while inhibition of DNA synthesis is also observed.[2] As opposed to vancomycin and metronidazole, cadazolid showed a strong inhibition of *C. difficile* toxin production and spore formation in vitro. In a human gut model of CDAD, cadazolid demonstrated potent activity against hypervirulent *C. difficile* while sparing most of the normal gut microflora.[3] In Phase 1 studies, this compound was well tolerated with very low systemic exposure and high concentration in the colon.[4] Following successful completion of a Phase II study in CDAD where cadazolid showed clinical cure rates similar to vancomycin while having lower recurrence rates,[5] cadazolid is now in Phase III clinical development.

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LE056 | Cyclic Peptides: From Nature to the Laboratory

Fernando Albericio[1,2]

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2) School of Chemistry, Yachay Tech University, Urcuquí, Ecuador

Peptides have experienced a remarkable renaissance as therapeutic agents in recent years. They are situated between small molecules (<1000 Da) and proteins, two of the most extensive classes of well-established therapeutic agents. Peptides provide both the specificity and potency of larger protein biologics but with zero or low immunogenicity. Furthermore, they are smaller, more accessible and cheaper to manufacture using chemical methods, thus presumably combining the advantages of the two therapeutic approaches. While nature has been fine-tuning the bioactive chemical structure of these structures for thousands of years, peptide chemists and protein engineers have the exciting challenge of improving the intrinsically unfavorable pharmacokinetic properties of the majority of native peptides. The drawbacks of peptides as therapeutic agents are associated with their generally high conformational instability. First nature and then scientists have seen cyclization as the best way to prepare constrained peptides with higher activities and improved pharmacokinetic properties. In this presentation, we will review our current research devoted to the synthesis of natural cyclic peptides (pipecolidepsin, baringolin…), as well as the design and synthesis of cyclic peptides with improved properties.

LE057 | Successful Drug Discovery and Development Case Studies of Protein Epitope Mimetics

Daniel Obrecht,[1] Sophie Barthélémy,[1] Christian Bisang,[1] Klaus Dembowsky,[1] Eric Chevalier,[1] Deniau Deniau,[1] Frank Gombert,[1] Francoise Jung,[1] Alexander Lederer,[1] Guillaume Lemercier,[1] Christian Ludin,[1] Anatol Luther,[1] Kerstin Moehle,[2] John A. Robinson,[2] Barbara Romagnoli,[1] Manuella Schmidt-Billet,[1] Francoise Jung,[1] Odile Kessler-Sellier,[1] Steffen Weinbrenner,[1] Johann Zimmermann[1]

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Protein epitope mimetics (PEM) technology has emerged as a powerful cyclopeptide-based approach to identify potent and selective modulators of protein-protein interactions (PPIs).[1] This presentation will describe successful case studies of applying the PEM approach from discovery to the clinic. POL6014 is a highly potent and selective inhibitor of human neutrophil elastase for inhaled application in alpha-1 antitrypsin deficiency (AATD) and cystic fibrosis (CF). POL7080 is a first in class novel anti-pseudomonal antibiotic with a novel mode of action.[2] and has started phase II. POL6326, a novel potent and selective CXCR4 antagonist, is the most advanced PEM molecule and currently in phase II clinical trials for stem cell transplantation and tissue repair.

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LE058 | Abstract unavailable at the time of printing

LE059 | Novel Inhibitors of Alzheimer's Associated Beta-Secretase—Clusters of Heparan Sulfate

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1) Victoria University of Wellington, The Ferrier Research Institute, Gracefield Research Centre, Lower Hutt, New Zealand
2) Center for Glycobiology, Institute of Integrative Biology, University of Liverpool, UK

Heparan sulfate (HS), a highly sulfated glycosaminoglycan, plays a crucial role in a range of essential physiological processes. Functions of HS depend on ionic interactions between its negatively charged sulfates and carboxylate groups with cationic regions of a variety of proteins such as cytokines, growth factors, lipases and proteases. HS has been identified as the first natural regulator of the cleavage of the amyloid precursor protein by the beta-secretase (BACE-1),[1] a protease responsible for accumulating the toxic Aβ peptides in Alzheimer's disease.

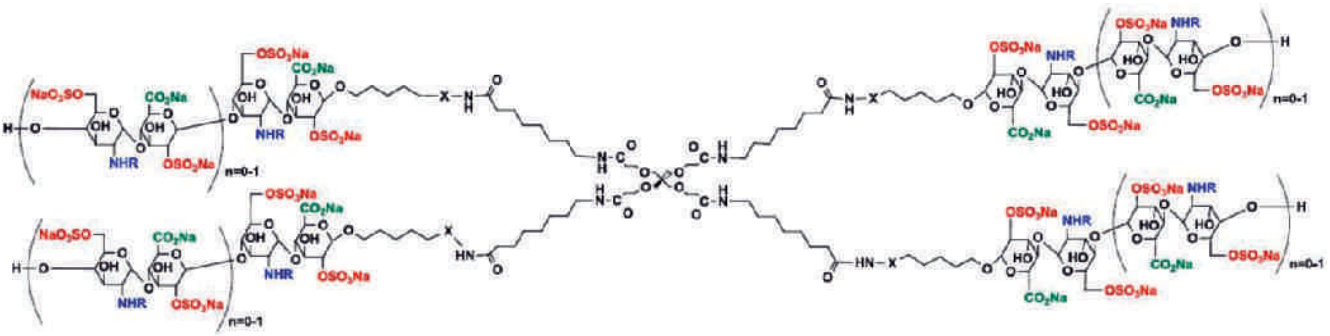
The complex synthesis of HS oligosaccharides (octa- to dodecasaccharides) has been investigated by a number of groups, including ours,[2,3] but despite many useful modifications and improved glycosylation protocols, multi-step syntheses of HS targets remain cumbersome and costly. We aimed to greatly simplify the synthesis requirements while retaining the desired bioactivity of HS structures.

Here, we report the synthesis of a novel targeted library of HS clusters with varied carbon chain linkers and small specific HS structures that are capable of mimicking long polysaccharide chains of natural HS. Flexible branches of dendritic scaffolds have been end-capped with more readily synthesized HS sequences (di- and tetrasaccharides) within controlled spatial parameters. These di-, tri- and tetravalent mimics can reach the size of multi-generational cascade molecules achieving strong yet reversible 'Velcro' type interactions.

The highly negative charge density of sulfated HS fragments causes very strong electrostatic repulsion of such species that attach to the dendritic scaffold. This usually gives complex mixtures with only partial end-capping of multiple branches. However, our robust and scalable chemical coupling method[4] we have recently developed has solved this problem, enabling us to greatly improve reaction yields and to access pure well-defined single HS cluster entities. We have examined how the variations in the degree of sulfation of surface sugars and the lengths and number of chain linkers triggered changes in bioactivity.

Clusters of HS have been shown to be inhibitors of BACE-1[4] (IC_{50} (μg/mL) 0.02–0.006; Heparin – IC_{50} (μg/mL) 0.002) and had no anticoagulant activity. Unlike heparin, such synthetic compounds would thus be expected to have no significant side effects related to anticoagulation. These have also been checked for ex vivo activity in a mouse brain slice assay which replicates many aspects of the in vivo context, crucially including bioavailability.

The most active derivatives identified in this study provide attractive lead compounds for the preparation of potent inhibitors for BACE-1, which may find use as therapeutic agents for Alzheimer's disease.[3,4]



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LE060 | Exploring the Dynamics of Ligand–Protein Interactions Using SPR Biosensors

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Biosensors with SPR detection are well suited for a broad range of applications within the life sciences, enabling a versatile experimental design and information rich output. The technology can also be used throughout the drug discovery process, from target validation to screening and lead optimization. This sensitive and versatile biophysical technique for interaction analysis complements high throughput methods and is transforming the drug discovery process by providing detailed and dynamic information about molecular interactions. The presentation will focus on the application of SPR biosensor technology for analysis of complex and dynamic molecular interactions.

The first steps involve the validation of sensor surfaces and establishing basic characteristics of the proteins thought to be suitable as drug targets. This is followed by the identification of suitable experimental design and data analysis strategies for efficient identification of hits. Identified hits are validated by a number of techniques, including distinction between specific interactions to a defined binding site and weaker nonspecific interactions to other sites or the general protein surface. The identification of the binding site of interest, the stoichiometry and the basic characteristics of the interaction are defined on the level of initial hits and the proteins used for screening. Methods for more detailed characteristics of hits/leads and the use of analogues series, protein variants and modifications of experimental conditions will be illustrated. A variety of examples will be used to demonstrate how SPR biosensor-based strategies are implemented for lead discovery.

LE061 | Breaking the Equilibrium Dissociation Constant into Fragments: The Use of Binding Kinetics and Thermodynamics in the Development of Selective Ligands

Iwan de Esch

VU University Amsterdam, Medicinal Chemistry, De Boelelaan 1083, 1081 HV, Amsterdam, The Netherlands

Fragment-based lead discovery (FBLD) represents a highly efficient and design-driven approach to interrogate protein targets and to develop biologically active compounds. Identified hit fragments (molecules with molecular weight lower than 300 Da) are linked, merged or grown into bigger ligands. The efforts are guided by structural information of the protein target that is obtained by biocomputational studies or X-ray crystallography. Most often, biochemical assays and equilibrium dissociation constants are being used to assess the quality of interaction between the fragment or ligand with the protein.

However, there is a growing number of reports, including from our lab, that indicate that the equilibrium dissociation constant does not always give accurate insights in the ligand–protein recognition process. Indeed, the kinetic components (k_a and k_d) and the thermodynamic parameters (including ΔH and ΔS) can lead to very different insights. For example, two compounds with comparable equilibrium dissociation constant can have a very different binding kinetics profile. It has also been shown that thermodynamic analysis provides information on ligand binding modes that the equilibrium dissociation constant cannot provide. Both kinetic and thermodynamic binding profiles have been used to detect subtle differences in binding of a ligand to two homologous proteins, offering a new opportunity to design selective ligands.

This presentation will discuss our efforts to incorporate binding kinetics and thermodynamics in the FBLD process. By developing structure–kinetic relationships (SKR) and structure–thermodynamic relationships (STR) next to the classic structure–activity relationships (SAR), and combining this data with structural information (obtained from biocomputational studies and X-ray crystallography), new insights will be obtained with respect to ligand–protein interactions.

LE062 | Medicinal Chemistry Optimisation of Binding Kinetics

Mike Waring

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A significant proportion of drugs reaching the market exhibit nonequilibrium binding characteristics.[1] Intuitively, it is likely that this represents a significant enrichment in compounds of this type the proportion of such compounds entering development, and so these compounds must have benefits with respect to attrition. This could be due to increased efficacy and/or reduced toxicity. However, the majority of them appear to have been discovered without knowledge of their kinetic behaviour at the time of compound selection and these properties were revealed later as they became of greater interest to the wider community and subject to more detailed studies.

It is often stated that association and dissociation rates cannot be manipulated rationally during optimisation. This talk will cover some of the principles of attempting to do so, highlighted with literature examples and data generated through our ongoing collaborative research project “Kinetics for Drug Discovery” (K4DD).[2] From these examples, some general observations give rise to hypotheses that may be of general relevance to the manipulation of kinetics and to a more detailed understanding of the underlying processes driving these observations at a molecular level.

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LE063 | CRTh2: Can Residence Time Help?

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The measurement of ligand binding potency is fundamental to medicinal chemistry.[1] However, simple potency data gives no indication of the underlying kinetics of the binding and unbinding processes. Binding events can occur over seconds, minutes, hours or days, and these differences can give rise to both desirable and undesirable consequences (Figure 1).[2,3] The medicinal chemistry community is now embracing the phenomenon of residence time not only by unraveling the possible consequences of fast and slow kinetics, but by harnessing this extra dimension of ligand-target binding.

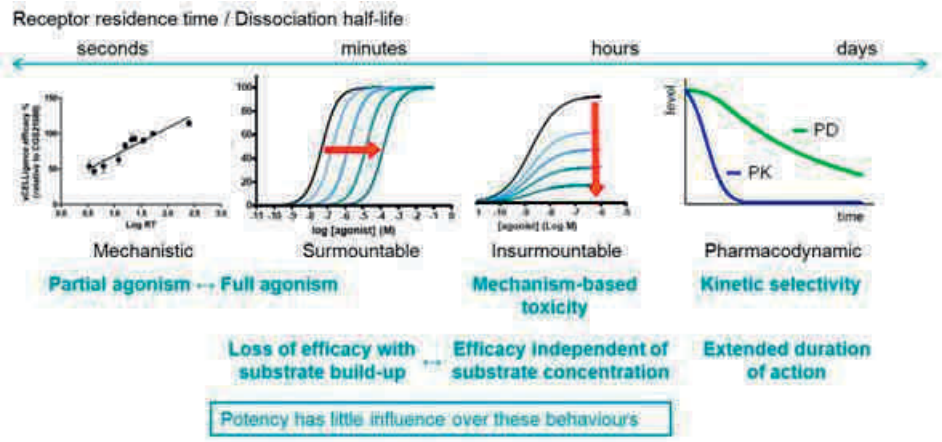

Figure 1. Possible consequences of short or long receptor residence times.

CRTh2 is a chemotactic receptor expressed on several inflammatory cell types. It is activated by prostaglandin D2 and several of its metabolites and thus forms part of the inflammatory component of the immune response. CRTh2 antagonists, to counter the pro-inflammatory responses to PGD2, have been the subject of intense research over the last decade, but despite over 15 compounds entering the clinic, attrition has been high and costly.

We sought an oral, milligram dose, once-a-day CRTh2 antagonist. This case study highlights our work to deliberately incorporate binding kinetics into our program strategy, to extend the duration of action long beyond that predicted by pharmacokinetics and ultimately lower the necessary dose. This talk also outlines the molecular origins of the slow kinetics, how structure–kinetic relationships (SKR) revealed the requirement of specific functional groups and how we harnessed this to design compounds with long dissociation half-lives of 24 h and above.

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LE064 | An Introduction to Ion Channels as Drug Targets

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The talk will be divided into two parts: 1) a general introduction to ion channels, and 2) why ion channels constitute an interesting class of drug targets.

Ion channels are integrated membrane proteins that secure the ability of exchange of charged ion between the cell and the extracellular environment across the lipophilic surface plasma membrane. The vast majority of ion channels allows the selective passage of only a single type of ions, the most prominent being K^+, Na^+, Ca^{2+} and Cl^-. The transport of ions via ion channels is a complete passive process and the direction of ion flow is determined by the charge and ion concentration differences between the intracellular and extracellular environment of the cell as described by the electrochemical gradient. In principle, sufficient ion flow via ion channels could be obtained by a single type of K^+, Na^+, Ca^{2+} and Cl^- channels but this is far from reality. Approximately 150 different genes coding for ion conducting α-subunits have been identified. The complexity of ion channels is further increased by a number of accessory non-conducting subunits and alternative splice variants. In addition, ion channel regulation can be accomplished by a number of different factors including voltage activation, various aspects of ligand activation and various types of phosphorylations. The overall picture of ion transport via ion channels thereby becomes relatively complex. In order to understand the resulting biological and physiological impact of ion channel activity a thorough understanding of regulation, biophysical characterization and interplay between ion channels is a prerequisite.

Ion channels have traditionally gained most attention in excitable tissue such as the central nervous system and the heart where a number of diseases have been associated to loss or gain of ion channels function. This has generated an independent category of “channelopathies” that is a common denominator of diseases related to any malfunction of ion channels. In addition to be important in

excitable tissue, ion channels also play a prominent role in epithelial tissue and is important for controlling water and salt homeostasis and secure secretion of various peptides and hormones including insulin.

From a drug development perspective ion channels have gained substantial interest due to their paramount importance in a number of physiological functions and their association to various diseases. Taken as a class of proteins ion channels are the second most common drug target across indications only surpassed by drugs targeting G protein-coupled receptors. When it comes to drug development ion channels are not easily accessible due to the complex nature of their activation and the low throughput in functional screening assay. State of the art for addressing ion channel function is a technique called patch clamp that, when performed manually, is very labor intensive. This notion is however changing these years due to development of new higher throughput screening systems based upon electrophysiological evaluation. A number of interesting opportunities are therefore to be pursued in the coming years.

LE065 | CNV1014802, A Novel $Na_V1.7$-Selective State-Dependent Sodium Channel Blocker for the Treatment of Chronic Pain: From Discovery to Phase 2 Clinical Study

Gerard M. P. Giblin

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Blockade of voltage-gated sodium channels is a clinically validated mechanism for the treatment of chronic pain. However, current drugs of this class are associated with inconsistent efficacy and are often poorly tolerated; hence, there is a need for new agents with an improved efficacy and safety profile. Recent human genetic studies have shown that patients with loss-of-function mutations in the gene encoding $Na_V1.7$ feel no pain, whereas patients with $Na_V1.7$ gain-of-function mutations show increased pain sensitivity. Consequently $Na_V1.7$ has attracted considerable interest as a target for drug development in pain. Trigeminal neuralgia is a relatively rare but debilitating facial pain condition thought to arise from compression of the trigeminal nerve. Patients experience a searing, electric shock-like pain occurring in attacks known as paroxysms, which are often triggered by normal daily activities. Treatment options for this highly painful condition are limited: current drugs have limited efficacy and are poorly tolerated. We report the discovery and development of a novel state-dependent $Na_V1.7$ blocker, CNV1014802, for the treatment of trigeminal neuralgia and other chronic pain conditions. CNV1014802 has an excellent preclinical profile and has successfully completed Phase 1 trials. An innovative Phase 2a study in trigeminal neuralgia patients will be completed in 2014. The discovery of CNV1014802 and its preclinical and early clinical profile will be presented.

LE066 | Pain-Relieving Peptides Targeting Acid-Sensing Ion Channels

Eric Lingueglia

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Acid-sensing ion channels (ASICs) are excitatory proton-gated and voltage-independent cation channels widely expressed in the central nervous system and in peripheral sensory neurons. They have been associated with nociception, fear, depression, seizure, and neuronal degeneration, suggesting roles in pain, neurological and psychiatric disorders.[1]

We have identified from animal venoms several peptide blockers of these channels, and we are using these specific inhibitors, in combination with in vivo gene silencing and knockout mice, to unveil the role of different ASIC channel subtypes in the pain circuits.[2] Mambalgins are new three-finger peptides recently discovered by our group from the deadly venom of the African snake black mamba,[3] which have the property to specifically inhibit several subtypes of ASIC channels. These peptides exert strong analgesic effects in vivo in mice upon both central and peripheral injection. The central analgesic effect is supported by heteromeric channels made of ASIC1a+ASIC2a subunits, while the peripheral effect involves ASIC1b-containing channels. Interestingly, the analgesia evokes by intrathecal injection can be as potent as morphine but is resistant to naloxone and does not involve opioid receptors. Mambalgins are devoid of apparent toxicity in mice and seem to produce less unwanted side effects than morphine. These peptides bind to the closed state of the channel and decrease the apparent affinity for protons. They bind into the pH sensor (i.e., the acidic pocket involved in pH sensing) in the extracellular region of ASIC and interact with at least three different domains of the channel to exert both stimulatory and inhibitory effects, eventually trapping ASIC in the closed conformation.[4]

Naturally occurring inhibitory peptides like mambalgins have thus been very important to identify different ASIC channel subtypes as new potential therapeutic targets for pain in the central (ASIC1a, ASIC1a+ASIC2a) and the peripheral (ASIC1b, ASIC3) nervous system. Their potent analgesic effects in animal also illustrate the potential therapeutic value of several of these peptides.

Acknowledgements:

Supported by CNRS, INSERM, the Fondation pour la Recherche Medicale (FRM), the Agence Nationale de la Recherche (ANR) and the Association Française contre les Myopathies (AFM).

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LE067 | Discovery of Ivacaftor (VX-770), a CFTR Potentiator for the Treatment of Cystic Fibrosis in G551D Patients

Sabine Hadida

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Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disorder in Caucasian populations, affecting approximately 70,000 patients worldwide. CF is caused by decreased chloride transport across epithelial tissues due to defective or deficient CFTR proteins, which result from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Currently, over 1900 CFTR mutations are known. The *G551D* mutation is a gating mutation that is present in 4–5% of CF patients. High-throughput screening (HTS) was used to identify pharmacological agents that modulate CFTR protein activity located at the cell surface, leading to enhanced chloride transport. Using the resulting HTS hits, extensive medicinal chemistry, structure–activity relationship analyses and formulation efforts were carried out and resulted in the discovery and development of ivacaftor (VX-770). Ivacaftor is now approved for treatment of CF patients aged 6 years and older who have the *G551D* and certain other CFTR mutations.

LE068 | Cebranopadol, A Novel, Potent Analgesic

Henning Steinhagen

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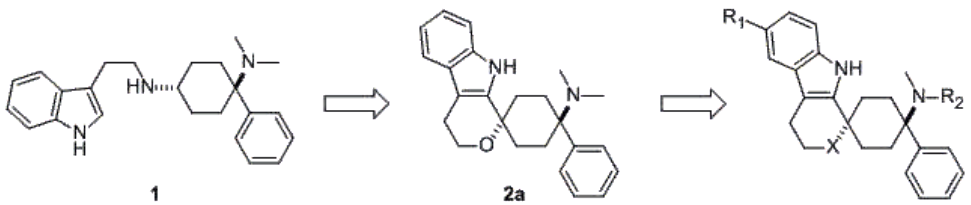
In this communication, we will disclose for the first time the discovery of cebranopadol (**3a**), a novel, highly potent and orally available analgesic. Cebranopadol, which acts as a nociceptin/orphanin FQ peptide (NOP) and opioid receptor agonist, is currently undergoing clinical trials for the treatment of severe chronic nociceptive and neuropathic pain. The discovery of **3a** is based on uncyclized indole diamine derivatives **1**, which were subsequently converted to novel spirocyclic derivatives **2a**. Based on the promising scaffold **2a**, a full lead optimization project was initiated. Optimization parameters comprised both functional NOP and opioid receptor data as well as PK and preclinical in vivo models of acute and neuropathic pain. The derived structure–activity relationships from medicinal chemistry optimization efforts including the selection and further characterization of cebranopadol will be described. Key for the selection of this innovative drug candidate were efficacy and tolerability data compared to standard opioids.



3a, X = O, R_1 = F, R_2 = Me
(Cebranopadol)

LE069 | Discovery of BAY 1002670—A Highly Potent and Selective Progesterone Receptor Modulator for Gynecological Therapies

Wolfgang Schwede, Wilhelm Bone, Arwed Cleve, Martin Fritsch, Ulrich Klar, Carsten Moeller, Andrea Rotgeri, Andrea Wagenfeld

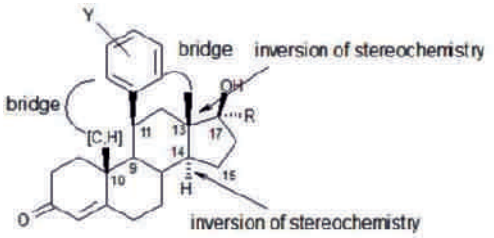
Bayer Pharma AG, Global Drug Discovery, Muellerstr. 178, 13353 Berlin, Germany

Progesterone receptor (PR) modulating ligands have shown clinical utility in a range of potential indications such as uterine fibroids, endometriosis and breast cancer. Despite of the discovery of the first PR antagonists more than 30 years ago, so far no compound has

been approved in any indication for long-term application. Among other reasons, suboptimal selectivity and insufficient safety profiles of previous candidates have led to discontinuation of development programs.

As result of an intensive optimization program at Bayer, BAY 1002670 was identified as a highly potent and selective PR modulator. The 11beta-phenyl group was found to be the crucial structural element of all potent steroidal PR modulators. Besides effects on the PR, early 11beta-substituted compounds showed strong antagonistic effects towards the glucocorticoid receptor (GR). For long-term applications to treat chronic conditions, an improvement of selectivity was considered to be essential. Therefore, the selectivity of early PR modulators has been optimized by systematic modifications in numerous learning cycles. Several variations of the steroid skeleton have been studied. Since the *para* substituent (Y, see Figure) of the 11beta-phenyl group and the 17alpha-side chain were both found to play a key role for potency and selectivity, these two positions have been the focal points for optimization. The structure–activity relationship (SAR) explored will be presented.

The potency and selectivity of BAY 1002670 was assessed in relevant in vitro and in vivo models. Furthermore, the efficacy of BAY 1002670 in pre-clinical disease models was demonstrated. BAY 1002670 is currently under clinical evaluation for the treatment of gynecological disorders.



Modification of 11 β-aryl steroids:
- broad modification of R and Y
- inversion of stereochemistry at C-13 and C-14
- introduction of Δ^{14} or Δ^{15} double bond
- 10β-H
- 10β-methyl
- $\Delta^{9(10)}$ double bond
- 10,11-bridge
- 11,13-bridge

LE070 | Design, Synthesis and Biological Evaluation of Potent and Selective Class IIa Histone Deacetylase Inhibitors as a Potential Therapy for Huntington's Disease

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Inhibition of class IIa HDAC enzymes have been suggested as a therapeutic strategy for a number of diseases, including Huntington's disease. Class IIa HDACs are large proteins with multiple functions including transcription factor binding and *N*-acetyl lysine recognition. The ability of small molecules to replicate the beneficial effects of the class IIa HDAC genetic suppression studies in preclinical models via occupancy of the class IIa HDAC catalytic domain would provide a rationale for small-molecule therapy. Guided by crystallography and structure-based design, we developed class IIa-selective tri- and tetra-substituted cyclopropane scaffolds by exploiting a lower selectivity pocket that is characteristic for the class IIa HDACs, and not present in class I and class IIb HDAC subtypes. Selected inhibitors were co-crystallized with the catalytic domain of human HDAC4, which confirmed the existence of the selectivity pocket and formed the basis for further optimization. Our crystal structures reveal a "closed-loop" form of the HDAC4 catalytic domain.

These molecules exhibit a very different selectivity profile from that of SAHA, and other previously studied HDAC inhibitors, and exhibit selectivity over the class I HDAC isoforms that are actively involved in chromatin remodeling and transcriptional regulation. Molecules were identified with good brain and muscle exposure following oral administration, which should enable the assessment of their therapeutic benefit in both peripheral and CNS disorders. These selective inhibitors are suitable as a means for evaluating potential efficacy and proof of concept studies in preclinical disease models in vivo where deregulation of class IIa HDAC biology has been implicated.

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LE071 | Structure-Guided Design of Potent, Selective, Orally Bioavailable Tankyrase Inhibitors

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Recent discoveries have implicated tankyrase (TNKS1/2) as a potentially useful target for the treatment of APC-mutant colorectal cancer and other cancers in which the Wnt signaling pathway is over-activated. Our co-crystal structure of TNKS1 with the known small-molecule TNKS1/2 inhibitor IWR2 revealed a novel binding mode and provided key insights into the selectivity profiles of known PARP1/2 and TNKS1/2 inhibitors. This structural data was used in combination with data obtained from various hit molecules discovered through our high-throughput TNKS1 auto-PARsylation screen to facilitate the development of two novel drug-like lead series with distinctly different binding modes. Independent optimization efforts in each series afforded highly potent, selective, orally bioavailable leads. BID dosing of these leads in a 3-day mouse DLD-1 tumor pharmacodynamic model led to robust, dose-dependent accumulation of axin and inhibition of transcription. In 8-day continuous dosing studies in naïve mice, we confirmed that doses at which we observed pharmacodynamic effects in the 3-day DLD-1 tumor models were well tolerated. The leads presented herein are expected to be of considerable utility in future in vitro and in vivo studies aimed at elucidating the potential of selective pharmacological tankyrase inhibition in cancer therapy.

LE072 | Novel Potent Inhibitors of the Histone Demethylase KDM1A

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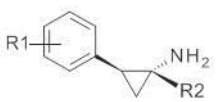
In eukaryotic cells, DNA is organized and packaged into higher order structures known as chromatin. The organization of chromatin is dynamically regulated by several post-translational histone modifications, including acetylation, phosphorylation and methylation. Altogether the combination of these modifications coordinates several relevant cellular processes including transcription, DNA replication, DNA repair and genomic stability. The inhibition of histone lysine specific demethylases is regarded as a useful strategy to alter chromatin structure, with the aim of interfering on the cellular mechanisms behind cancer cell growth. KDM1A is a FAD-dependent amine oxidase, having histone H3K4me1/2 as substrates, found in various transcriptional co-repressor complexes. High expression of KDM1A and its correlation with poor prognosis reported in neuroblastoma, prostate cancer and non-small-cell lung cancer, as well as its high expression reported in acute myeloid leukemia, strongly suggest a role of this histone demethylase in cancer. As a result, KDM1A has been increasingly recognized as an attractive therapeutic target in oncology.

At the Drug Discovery Program of the European Institute of Oncology, epigenetic projects are highly prioritized, and our interest in KDM1A has been directed both to the search of irreversible as well as reversible inhibitors. Among the many known mono-aminooxidase (MAO) inhibitors screened for KDM1A inhibition, tranylcypromine (TCP) often emerged as a moderately active hit, which irreversibly binds to the FAD cofactor. In the domain of covalent inhibition mechanism, various series of TCP-based compounds have been developed, leading to potent inhibitors where the decoration of the residues bound to the cyclopropyl core was mainly applied.

Here, we report a new, potent series of KDM1A inhibitors, characterized by the presence of various substituents at the 1 position of the amino-cycloproyl moiety of TCP. A versatile and scalable synthetic process has been developed for the synthesis of these compounds, which allows the introduction of a large set of diverse substituents. The stereoselective synthesis applied allowed to obtain the optically active *trans* isomers of the cyclopropane ring. The expansion of this series led to the identification of inhibitors with optimized potency and ADME characteristics, able to inhibit the target after in vivo administration and, importantly, to determine a significant survival increase in murine model of promyelocitic leukemia. Crystal structures are presented for representative compounds, confirming their irreversible mechanism of inhibition, through the formation of covalent adducts with FAD, and elucidating the effect of stereoisomerism on KDM1A inhibition. In quest for reversible inhibitors, we screened the diversity set of our chemical collection (34000 compounds) in an HTS campaign, which successfully led to the identification of hits with IC_{50} values in the low micromolar range belonging to five chemical classes. An intensive effort of hit expansion and optimization, guided by structure-based drug design, is ongoing.



LE073 | Functional and Structural Insights into GPCR Allostery

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It is now widely accepted that G protein-coupled receptors (GPCRs) are highly dynamic proteins that adopt multiple active states linked to distinct functional outcomes. Furthermore, these states can be differentially stabilised not only by orthosteric ligands, but by allosteric ligands acting at spatially distinct binding sites.[1] The key pharmacological characteristics of GPCR allostery include improved

selectivity due to either greater sequence divergence between receptor subtypes and/or subtype-selective cooperativity, a saturability of effect, probe dependence (whereby the magnitude and direction of the allosteric effect change with the nature of the interacting ligands), and the potential for biased signaling.[2] At the mechanistic level, these properties reflect the ability of allosteric ligands to change the interactive properties of a GPCR towards orthosteric ligands and/or intracellular effectors. Two key challenges now facing the field include the ability to detect and quantify allostery and bias in a manner that can inform structure–activity relationships (SAR), and the delineation of the structural basis of allostery and bias. With respect to the former challenge, we have combined analytical pharmacological modeling with chemical biology approaches to demonstrate that clear, albeit often subtle, changes in SAR can have profound and often divergent effects on biased agonism and allostery for different classes of GPCRs.[3–5] We have also rationally designed bitopic ligands, which concomitantly bridge orthosteric and allosteric sites, to engender biased agonism.[6] With respect to the latter challenge, we have utilized structural, computational and pharmacological approaches to dissect the molecular mechanisms underlying allosteric modulation by positive and negative modulators of the muscarinic acetylcholine receptor family, which remains an important model system for understanding general principles of GPCR allostery.[7–8] We find that cooperativity can arise from a combination of stabilization of specific receptor states by an allosteric ligand and intramolecular communication between binding pockets within a given receptor state. These findings offer the potential for more rational design of the next generation of biased and allosteric ligands, as well as a general framework that can be incorporated into drug candidate selection matrices.

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LE074 | Discovery of the mGlu5 Negative Allosteric Modulator Basimglurant (RO4917523)

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mGlu5 negative allosteric modulators (NAMs) have emerged as novel approach for treating psychiatric indications including depression, fragile X syndrome, anxiety, obsessive-compulsive disorders and levodopa induced dyskinesia in Parkinson disease. Several mGlu5 NAMs are or have been in clinical development. In this presentation we will present the optimization process starting from a HTS hit leading ultimately to the development candidate basimglurant, which is in phase II clinical studies for depression and fragile X. The imidazole derivate basimglurant is characterized by high in vitro and in vivo potency as well as an excellent preclinical safety profile. In addition, we will present the discovery of the structurally related CTEP, which is the first published mGlu5 NAM that has a long lasting action in rodents.[1,2]

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LE075 | Allosteric Protein Kinase Inhibitors for Neurodegenerative Disease Therapies

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Protein kinases have emerged as promising targets for many diseases. In fact, more than a dozen of protein kinase inhibitors are on the market for the treatment of different oncologic disorders. More recently, some other protein kinase inhibitors have entered clinical

trials for chronic inflammatory diseases and neurodegenerative diseases.[1] As the human kinome has around 500 genes that encode for more than 2000 different kinases, one of the present challenges in the field is selectivity. In this sense, allosteric modulation of protein kinases emerges as one of the most promising strategies to achieve this important goal.

We have a vast experience on the design and discover protein kinases inhibitors.[2,3] Recently, we have used different approaches to determine potential allosteric sites on the protein surface that together with virtual screening studies open the possibility to find new allosteric modulators of glycogen synthase kinase 3, a key target for many severe human disorders such as Alzheimer's disease, multiple sclerosis, and other neurological pathologies. Three different series of allosteric modulators will be presented here together with their biological activity in different cell and animal models. Some of these compounds are now in pharmaceutical development to reach clinical trials.

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LE076 | Antibodies as Tools to Enable Small-Molecule Drug Discovery

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In the quest for small-molecule drug candidates, there have been a number of different approaches used including: existing lead or drugs, natural products, high-throughput screening and more recently established powerful fragment-based drug discovery.[1] Here, we highlight the potential of antibodies as research tools to identify and/or validate new chemical leads in small-molecule drug discovery. It is believed that this approach could decrease the risk of failure in the search for new small-molecule drug candidates addressing protein–protein interactions.[2] Antibodies, which carry low chemical risk, can define and stabilize dynamic protein structures and also provide important insight into the design of small-molecule screening assays. Moreover, by holding the target protein in a number of biologically active conformations, new sites (in particular allosteric sites), which would otherwise be inaccessible, may become available for binding. The ability to capture the target protein in a specific conformation with high affinity for a significantly long time opens the possibility for a small-fragment-molecule screening.

As a proof-of-concept, a very dynamic protein, which is able to adopt a number of different conformations in solution making it a challenging target for small-molecule drug discovery, was chosen as a target protein. Initially, a panel of antibodies against the target protein was generated and surface plasmon resonance (SPR) and fluorescence resonance energy transfer (FRET) assays were used to identify six high-affinity, function-modifying antibodies (K_D values ranged between 3–10 pM) able to constrain the protein in six different conformations in X-ray crystallography, and these were selected for this study. Due to the low dissociation rate constants (10^{-5}–10^{-6} s^{-1}) of the antibodies, it became possible to develop an SPR-based binding assay to screen a small molecule fragment library (3000 fragments). In this assay format, binding of small-fragment molecules to the protein successfully captured in a particular conformation by an antibody was investigated. The initial SPR results and corresponding hit rate show promise, and the results demonstrate the selectivity of small-fragment molecules for each conformation with a hit rate ranging between 2–3.7% depending on the antibody.

In conclusion, the use of antibodies to hold target proteins in biologically active conformations may provide a powerful tool aiding the discovery of new small molecules for challenging targets.

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LE078 | Update on Chemotherapy of Tropical Diseases Caused by Protozoan Parasites

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Tropical diseases pose a significant health threat to large populations of our world. Protozoan parasites are responsible for about 20 diseases and belong mainly to the hemoprotozoans or the intestinal protozoans. The most important protozoan diseases are malaria, amebiasis, leishmaniasis, Chagas disease and human African trypanosomiasis (HAT) affecting together hundreds of millions of people

and causing death of approximately one million per year. Less important are other intestinal protozoa and free-living opportunistic protozoa which can become very problematic in immuno-compromised individuals.

What drugs are currently used for the treatment of the hemoprotozoans? For malaria a broad array of drugs is available; however, they only represent 3 or 4 classes of related molecules. Most antimalarials suffer from drug-resistant parasites. State of the art are artemisinin-based combination therapies but even they seem to lose efficacy in certain regions. The main threat for antimalarials is clearly emerging drug resistance, but also availability of the drugs and the price. Leishmaniases are a complex disease group ranging from benign cutaneous disease to fatal visceral disease.[1] Until recently antimonials were in use, today better tolerated and more efficient drugs are available, such as, miltefosine, paromomycin or liposomal amphotericin B and combinations of the three drugs. Treatment of Chagas disease mainly depends on benznidazole and the nitrofuran nifurtimox.[1] The main problem with the two drugs is the poor safety profile and the limited effect on chronic infections. HAT or sleeping sickness is mainly treated by NECT, a combination therapy of nifurtimox and eflornithine.[2] Safety and efficacy are good, but the administration with 14 i.v. infusions is challenging and the costs for this treatment are very high. For all these diseases new drugs are needed either to overcome the drug resistance problem or to provide effective and safe treatments to the affected populations. New products should also be easy to administer (oral application, short treatment duration) and be affordable.

By the turn of the millennium, new initiatives were created, product-development partnerships, to enable research and development for new drugs for such neglected diseases. The Medicines for Malaria Venture (MMV)[3] or the Drugs for Neglected Diseases initiative (DNDi)[4] are two of the major players who contribute to close the R&D gap and bring new products to the patients suffering from protozoan diseases. At the Swiss Tropical and Public Health Institute we established 20 years ago a Screening Center for protozoan parasites (today we even include helminths). In vitro assays are available for the major protozoans and rodent models for malaria, HAT and helminths. The Parasite Chemotherapy Unit collaborates with PDPs, academic groups and pharmaceutical companies. Several clinical candidates are currently in clinical trials which resulted from such collaborations, e.g. the synthetic peroxide OZ439 or the spiroindolone KAE609 for malaria or fexinidazole and the benzoxaborole SCYX-7158 for HAT.[5] The new molecules for HAT could massively add to the success of the campaign to eliminate HAT within the next 15 years.[6]

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LE079 | Antimalarial Trioxolanes—Exploiting Peroxide Reduction for Parasite-Selective Drug Delivery

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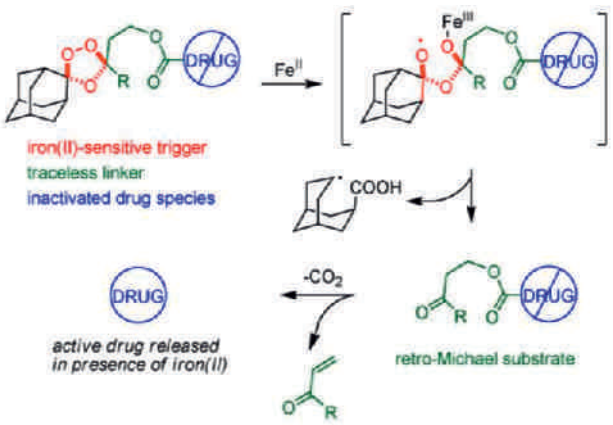
The action of peroxidic antimalarial agents including the artemisinins and trioxolanes is associated with initial reduction of the sterically hindered oxygen–oxygen bond. It is generally thought that peroxide cleavage is promoted by ferrous iron in the parasite digestive vacuole, although other mechanisms of reduction not involving iron have also been proposed. Our group is developing novel trioxolane moieties that exploit peroxide reduction to achieve parasite-targeted drug delivery. This has been accomplished by coupling peroxide reduction to drug release from a traceless linker. Effectively, these compounds 'diagnose' a malaria infection in situ, and release an active partner drug only following a 'positive' diagnoses. As such, this approach may be particularly well suited for future mass drug administration campaigns and/or in antimalarial prophylaxis, since in these contexts uninfected patients would be spared exposure to active drug. In this talk, we will present our efforts to validate ferrous-iron-dependent drug delivery in vivo, using activity-based probes to detect drug release in parasites and mouse tissues. We will also describe the stereoselective synthesis of a new generation of peroxide-based drug delivery systems with improved drug-like properties.



LE080 | Antimalarial Lead Discovery from Phenotypic Screening: Unraveling the Black Box

Francisco-Javier Gamo

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Malaria is a major global disease caused by parasites of the genus *Plasmodium* mainly affecting people living in the least developed countries. In 2012, more than 200 million cases of malaria were reported, resulting in approximately one million deaths. *Plasmodium falciparum* infection is the biggest cause of mortality although other malarias contribute significantly to the considerable humanitarian and economic burdens in malaria endemic regions.

Because parasites have developed resistance to all historically used antimalarials, the recommended first-line treatments for *P. falciparum* malaria are artemisinin-based combination therapies (ACTs). Over many years these drugs have proved to be highly efficacious although their effectiveness is now being compromised by resistance with recent evidences indicating that slow clearing *P. falciparum* infections is spreading in regions beyond the Thai–Cambodian border. Consequently, there is an urgent need for novel antimalarial drugs that can replace ACTs and provide future treatment options that offer advantages over the current standards of care.

To address the need for novel treatments, the malaria scientific community has conducted several high scale phenotypic screens. This approach has identified thousands of starting points for the discovery of new antimalarial drugs. At our department, we have profiled the entire GSK corporate screening collection (>2 million compounds) in a whole cell assay. This initiative created the Tres Cantos Antimalarial Set (TCAMS) which was published in Nature and comprises of over 13K novel hit compounds that are freely available to the global drug discovery community.

This presentation will show the different approaches and tools that GSK is using to identify the most promising starting points for lead optimization projects as well as the learnings we are finding in our search for new antimalarial therapeutic options.

LE081 | Improving Physiochemical Properties of a Lapatinib-Based Lead Compound that Targets *Trypanosoma brucei*

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Human African trypanosomiasis (HAT) is a neglected tropical disease that places 70 million people in sub-Saharan Africa at risk. The insect-borne pathogen *Trypanosoma brucei* causes about 10,000 new cases of this disease annually. Drugs for HAT lack oral bioavailability and efficacy, but are also expensive, toxic, and drug resistance is emerging.[1] Therefore, new small-molecule chemotherapeutics are needed to treat HAT and other neglected tropical diseases.

We recently reported that, despite a lack of canonical tyrosine kinases in *T. brucei*, the human epidermal growth factor receptor (EGFR) inhibitor lapatinib showed inhibition of cellular proliferation, with an EC_{50} value of 1.54 μM against *T. brucei.* Based on this observation, a structure–activity relationship (SAR) study of the lapatinib chemotype resulted in NEU-617, an inhibitor with an EC_{50} value of 42 nM against *T. brucei* and great selectivity over host cells. This inhibitor also showed marginal ability to control infection in a murine model of HAT following oral dosing. However, NEU-617 has poor physiochemical properties, with a high molecular weight (541), high lipophilicity (clog*P*=7.1), and high binding to plasma proteins (>99%). These properties lead to limited efficacy in vivo, and we anticipate this to also explain the low central nervous system (CNS) exposure that we observe.[2] In this report, we describe ongoing SAR studies that have been directed at improving these physiochemical properties, while maintaining the desirable potency and selectivity profile of the lead compound.

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LE082 | High-End Design for GPCRs: Combining Protein Structure, Biophysical Data and Computational Water Network Energies

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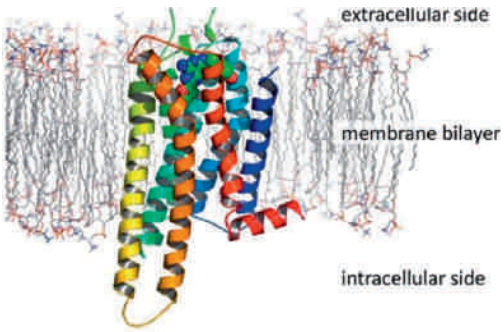
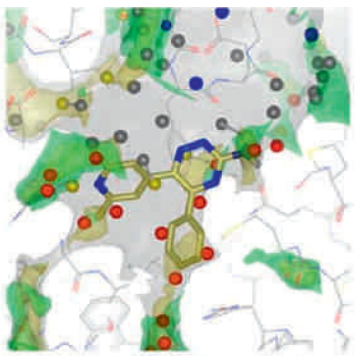
The recent availability of X-ray protein–ligand structures for a diversity of G protein-coupled receptors (GPCRs) in multiple conformations (inactive and active) enables rational drug design efforts to now be applied to this important drug target class. Insights from these new ligand–receptor complexes will be presented together with a variety of computational methods which are used to analyse druggability and drive design. Full structure-based drug design (SBDD) for GPCRs is now possible using a combination of advanced experimental and computational data; explicit water networks and their energetics, linked to lipophilic hotsots, are a critical 'third dimension' for SBDD, key for understanding ligand binding energies and kinetics.[1] Protein SBDD approaches, that have been highly successful with soluble targets such as kinases and proteases, can now be used to discover better GPCR drug candidates, including for the more "difficult" and previously undruggable GPCRs.[2]



GPCR topology ,exemplified by the crystal structure of A_{2A} StaR2 in complex with ZM241385 (PDB code 3PWH; simulated lipid bilayer


Druggable A_{2A} Antagonist site

StaR® proteins which have been thermostabilised in a chosen conformational state by the introduction of a small number of point mutations particularly enable these approaches since they are stable in detergent and can be readily removed from their native cell membranes and purified. StaR proteins can be used for biophysical screening techniques, fragment screening, and crystallisation to yield X-ray structures with both potent and weak ligands.

SBDD examples to yield clinical candidates with better properties and selectivity will be presented including adenosine A_{2A}, muscarinic M1 and orexin receptors. Binding site energetic surveys using GRID for lipophilic hotspots are found to be key drivers for binding. Progress and insights from the new structures for class B[3] and class C mGlu receptors will also be presented.

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LE083 | Nanobody-Enabled Fragment Screening on Active-State Constrained GPCRs

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In the past 10 years, GPCR drug discovery has relied on cell-based assays combined with high-throughput screening (HTS) of large compound libraries for lead discovery as well as optimization. However, progress in identifying new small molecule drugs has been disappointing and the pace of GPCR drug discovery is slow. One key problem is that compounds do not only need to target the correct GPCR, but the drugs must also exhibit the appropriate efficacy profile: agonist, partial agonist, neutral antagonist or inverse agonist. Even worse, hits from HTS screens frequently must be deconstructed to remove liabilities that cause toxicity or non-ideal ADME properties.

Fragment-based drug discovery uses low-molecular-weight, moderately lipophilic, and highly soluble fragments as starting points for developing novel drugs. FBDD is particularly advantageous for its ability to more completely assess "compound space" for molecules that interact with the target of interest. Last years, our lab has shown that nanobodies are effective tools for stabilizing agonist-bound active states of GPCRs.[1–3] Building on this technology, we have developed a nanobody-enabled fragment screening approach to explore new chemical space for the development of drugs targeting GPCRs. Our approach has the competitive advantage to other methods that we can screen fragments that exclusively bind to particular functional conformations of the receptor allowing us to triage our fragments

according to efficacy profile and potency from a single biophysical assay. Nanobody-enabled screening of a moderate sized fragment library of 1000 compounds led to the discovery of several fragments with an agonist efficacy profile.

References:

[1] *Nanobody stabilization of G protein coupled receptor conformational states,* J. Steyaert, B. K. Kobilka, *Curr. Opin. Struct. Biol.* **2011**, *21*, 567–572.
[2] *Structure of a nanobody-stabilized active state of the β2 adrenoceptor*, S. G. Rasmussen et al., *Nature* **2011**, *469*, 175–80.
[3] *Activation and allosteric modulation of a muscarinic acetylcholine receptor*, A. C. Kruse et al., *Nature* **2013**, *504*, 101–106.

LE084 | Covalent Agonists Facilitating Crystallization of GPCRs

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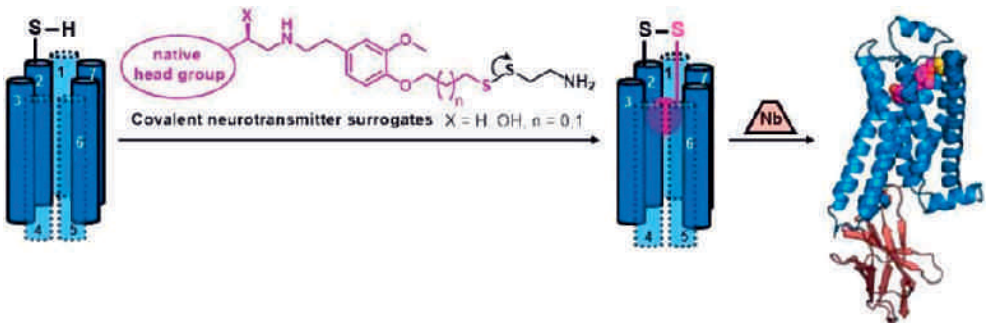
G protein-coupled receptors (GPCRs) constitute a large superfamily of target proteins (nearly 800 different human genes encode for GPCRs) and each of them can adopt functionally distinct conformations. The first X-ray crystal structures of druggable GPCRs in complex with ligands provide a basis for the investigation of molecular determinants responsible for affinity and selectivity of ligands.[1]



We have developed GPCR ligands as molecular probes for structural investigations and structure–function relationship studies. As an example, we established a disulfide-based methodology for the stabilization of a heterocyclic agonist bound low-affinity state of the β_2-adrenoreceptor leading to the first X-ray crystal structure of an agonist-bound GPCR.[2] Extending this strategy to GPCR-activating neurotransmitters and hormones, covalent catecholamines were constructed leading to the crystal structure of the β_2-adrenergic receptor (β_2AR) in complex with an irreversibly bound derivative of the endogenous ligand norepinephrine. Moreover, novel irreversible muscarinic acetylcholine receptor agonists led to the development of X-ray crystal structures of active state M2–agonist complexes.[3]

References:

[1] S. Granier, B. Kobilka, *Nat. Chem. Biol.* **2012**, *8*, 670–673.
[2] D. M. Rosenbaum, et al., *Nature* **2011**, *469*, 236–240.
[3] A. C. Kruse, et al., *Nature* **2013**, *504*, 101–106.

LE085 | Crystallographic Structures Enable the Discovery of Selective 5-HT$_{1B}$ Receptor Ligands through Virtual Screening

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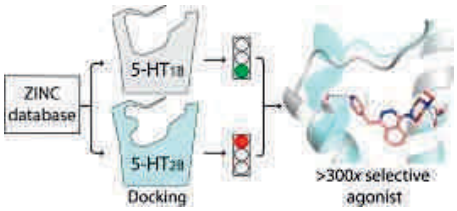
G Protein-coupled receptors (GPCRs) are grouped in receptor families that have evolved to recognize the same ligand. However, the structural similarities among the binding sites of these also lead to difficulties in finding new chemical scaffolds that can selectively bind to specific receptors. The recent determination of crystallographic structures for the human 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors bound to ergotamine gave the opportunity to understand the molecular determinants of ligand binding to these closely related receptors.[1] The molecular modeling field was challenged to submit models of these ligand–receptor complexes right before the release of the crystallographic coordinates in the context of the community-wide assessment GPCR Dock 2013.[2] In our participation in this challenge, we employed a ligand-guided homology modeling approach that combines the incorporation of experimental data from different sources and extensive molecular docking screening in an iterative manner.[3] Our best solutions were close to the experimental structure (ligand RMSD < 1.8 Å) and were among the most accurate of the assessment; they achieved the best and fifth-best predictions for 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors, and presented the best descriptions of the binding sites and ligand-receptor contacts.

Recent breakthroughs in GPCR structural biology have enabled the successful use of structure-based approaches in ligand discovery for several pharmaceutically relevant receptors.[4] We took advantage of the crystal structures of the 5-HT_{1B} and 5-HT_{2B} receptors to identify subtype-selective ligands. From docking screens of 1.3 million compounds, 22 molecules were predicted to be selective for the 5-HT_{1B} receptor over the 5-HT_{2B} subtype, a requirement for safe serotonergic drugs. Nine compounds were experimentally verified as 5-HT_{1B} selective ligands with up to 300-fold higher affinities for this subtype. Three of the most novel and selective ligands were agonists, representing new lead candidates against migraine.[5] Analysis of our best homology models of the two 5-HT receptors submitted to the GPCR Dock 2013 assessment revealed that despite their accuracy, they could not capture the critical structural features responsible for ligand selectivity. Our results demonstrate that structure-based screening is a fruitful approach to guide the discovery of ligands and suggest novel methodological approaches for modeling GPCR–drug complexes with improved accuracy.



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[2] *Advances in GPCR modeling evaluated by the GPCR Dock 2013 assessment: meeting new challenges.* Kufareva I., Katritch V., GPCR Dock 2013 participants, Stevens R.C., Abagyan R., *under review*.
[3] *Strategies for Improved Modeling of GPCR-drug complexes: Blind predictions of Serotonin Receptors Bound to Ergotamine.* Rodríguez D., Ranganathan A., Carlsson J., *under review*.
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LE086 | Pharmaceutical Protein and Peptide Engineering: From Once Daily to Once Weekly GLP-1 Dosing

Jesper Lau

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Proteins and peptides play unique roles in human physiology and are thus excellent templates for drug discovery. However, endogenously secreted proteins/peptides have many limitations when translated directly into pharmaceuticals. Poor half-life, bioavailability, pharmacodynamics, chemical and biophysical stability are common parameters that need to be significantly improved in order to transform a protein/peptide into a convenient drug. Tailored engineering of GLP-1 will be discussed based on Novo Nordisk case stories.

LE087 | 1,1'-*Spiro*-Substituted Hexahydrofuroquinoline Derivatives as Potent and Polar Cholesteryl Ester Transfer Protein (CETP) Inhibitors

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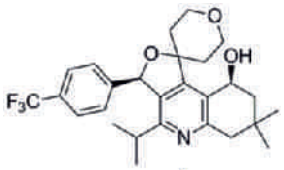
Cholesteryl ester transfer protein (CETP) is a glycoprotein that mediates the exchange of cholesteryl ester from high-density lipoprotein-cholesterol (HDL-C) with triglycerides from apolipoprotein B-containing lipoproteins. The net effects of this exchange are decreased low-density lipoprotein-cholesterol (LDL-C) levels and increased HDL-C levels. Epidemiological studies have linked increased levels of HDL-C with a decreased number of cardiovascular events. Inhibition of CETP could therefore answer the key question of whether raising HDL-C translates directly and on top of current treatments into a reduced risk for cardiovascular events.

Due to the hydrophobic nature of the CETP binding pocket, it is of no surprise that all published CETP inhibitors are highly lipophilic. In an effort to reduce the development risk of potential CETP inhibitors, a series of hexahydrofuroquinoline derivatives exhibiting potent CETP inhibition and reduced lipophilicity was identified. A practical synthetic route was developed and utilized for the synthesis of modifications. Initial SAR revealed that polar substituents were tolerated in various positions. Based on its in vitro properties and its PK profile, CETP inhibitor **1** was advanced into evaluation in pharmacodynamic models. Compound **1** demonstrated dose-dependent CETP

inhibition, and HDL-C elevation in hCETP transgenic mice and robust LDL-C reduction in hCETP/hApoB-100 mice. In contrast to the clinical CETP inhibitor anacetrapib, compound **1** eliminated completely from tissues of hCETP transgenic mice 21 days after cessation of treatment for 5 days at a fully efficacious dose. Compound **1** also showed no significant effects on in vitro aldosterone secretion and no significant effects on blood pressure and ECG parameters in telemetrized cynomolgus monkeys. This profile led to the selection of CETP inhibitor **1** as a development candidate. Using an improved synthetic sequence the tractable SAR within the series was used to bring up follow-up CETP inhibitors with further increased polarity and potency.



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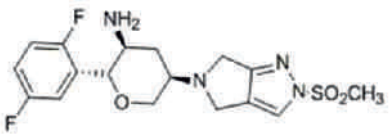
LE088 | Omarigliptin: A Once-Weekly Oral Antidiabetic Agent

Tesfaye Biftu, Ranabir Sinha-Roy, Ping Chen, Xiaoxia Qian, Dennis Feng, Jeffrey T. Kuethe, Giovanna Scapin, Ying Duo Gao, Youwei Yan, Davida Krueger, Annette Bak, George Eiermann, Jiafang He, Jason Cox, Jacqueline Hicks, Kathy Lyons, Huaibing He, Gino Salituro, Sharon Tong, Sangita Patel, George Doss, Aleksandr Petrov, Joseph Wu, Shiyao Sherrie Xu, Charles Sewall, Xiaoping Zhang, Bei Zhang, Nancy A. Thornberry, Ann E. Weber

Merck & Co., Inc., Whitehouse Station, NJ 08889, USA; E-mail: Tesfaye_Biftu@Merck.Com; Tel.: 732-594-6514

Omarigliptin (MK-3102) was identified as a potent and selective dipeptidyl peptidase 4 inhibitor with an excellent pharmacokinetic and safety profile amenable to once-weekly human dosing and selected as a clinical development candidate. This presentation summarizes the mechanism of action, scientific rationale, medicinal chemistry, pharmacokinetic/pharmacodynamic properties and human efficacy data for omarigliptin, which is currently in phase III clinical development.



Omarigliptin

References:

T. Biftu et al., *J. Med. Chem.* **2014**, *57*, 3205−3212.

LE089 | Discovery of GPR103 Ligands Demonstrating the Anorexigenic Effect In Vivo of GPR103 Antagonism

Anneli Nordqvist,[1] Jennie Georgsson,[1] Fredrik Bergström,[1] Martin J. Watson,[2] Charles D. Blundell,[2] Dorota Kakol-Palm,[1] Lisbeth Kristensson,[3] Kristina Nilsson,[1] Peter Brodin,[1] Anette Svensson Henriksson[1]

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2) C4X Discovery Ltd, Ducie House, 37 Ducie Street, Manchester M1 2JW, UK
3) Discovery Sciences, AstraZeneca R&D Mölndal, 431 83 Mölndal, Sweden

GPR103 is a family A G protein-coupled receptor. Activation of the receptor by the endogenous neuropeptide ligands, QRFP26 and QRFP43 has been reported to demonstrate an orexigenic effect.[1] QRFP26 and QRFP43 belong to a large family of biologically active peptides with a common C-terminal Arg-Phe-NH_2 motif. The C terminus of QRFP26 and QRFP43 has been demonstrated in a structure–activity relationship (SAR) study to be important for biological activity.[2] Given the increase in food intake and body weight by central administration of GPR103 agonists, antagonists of GPR103 have a potential use in clinic to target weight management by modulation of appetite.

In our efforts to discover new GPR103 antagonists amenable for drug development, a new series of pyrrolo[2,3-*c*]pyridines is described. These compounds display GPR103 affinity and functional antagonism with key compounds displaying good DMPK and safety parameters. In addition, a high throughput screening (HTS) was performed, where 17 new compound classes were discovered.[3] Three representative compounds, from three different clusters identified in the HTS, are presented. SAR, indicative of pharmacophore elements important for GPR103 affinity, is discussed for the pyrrolo[2,3-*c*]pyridines and one of the HTS clusters. Data from a pre-clinical obesity model measuring food intake over a three day study will be presented.

Given the importance of the C terminus for the biological activity of the endogenous agonists, the 3D-conformational ensemble observed in solution of the C-terminal heptapeptide $QRFP26_{(20\text{-}26)}$ was determined by NMR. Low energy conformations of a pyrrolo[2,3-*c*]pyridines antagonist were compared to this experimental structure, displaying a possible overlay of pharmacophore features.

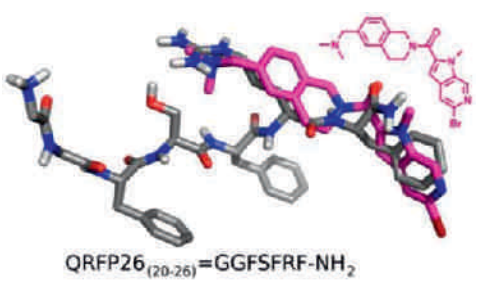

Figure 1. Overlay of an experimentally determined idealized conformation of $QRFP26_{(20\text{-}26)}$ and a pyrrolo[2,3-*c*]pyridine antagonist of GPR103.

References:

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LE090 | $Ca_V1.3$-Selective L-Type Calcium Channel Negative Allosteric Modulators. Novel Therapeutics to Slow the Progression of Parkinson's Disease

Richard B. Silverman

Department of Chemistry, Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, IL 60208-3113, USA

Parkinson's disease, a late onset neuropathology that is the second most common neurodegenerative disease, is characterized by tremors, bradykinesia, hypokinesia, and akinesia. The motor symptoms are attributable to the loss of neurons in the *substantia nigra pars compacta*, the part of the brain where the neurotransmitter dopamine, which is essential for control of movement, is released. It is not known why dopaminergic neurons in the *substantia nigra pars compacta* die in Parkinson's disease. Despite intense effort to identify neuroprotective agents for Parkinson's disease, there is nothing that even slows the progression of the disease.

It is now known that vulnerable dopaminergic neurons are unique in that they are calcium dependent, autonomous pacemakers. The calcium enters through L-type calcium channels (LTCCs) with a $Ca_V1.3$ pore-forming subunit. My collaborators have found that antagonism of this calcium channel with a nonselective LTCC antagonist, isradipine, a $Ca_V1.2$-selective antagonist used to treat hypertension, rejuvenates the neuron. Following numerous preclinical studies, isradipine completed phase II clinical trials and is entering phase III clinical trials, possibly this year. However, there are cardiovascular concerns and side effects with the use of this drug because of its nonselectivity toward LTCCs. To avoid the side effects of a nonselective antagonist, we have identified the first class of $Ca_V1.3$-selective antagonists, which have proven to be negative allosteric modulators of $Ca_V1.3$.

In this lecture, I will present the medicinal chemistry approaches taken to identify this new class of $Ca_V1.3$-selective compounds, including a homology model to identify the potential binding site, site-directed mutagenesis experiments to pinpoint the binding site, in vitro electrophysiology to demonstrate selectivity and possible mechanism of action, and preliminary in vivo animal studies to establish efficacy and safety for the treatment of Parkinson's disease.

LE091 | Restoration of Muscle Mass and Life-span in a Mouse Model of Spinal Muscular Atrophy by Molecular Correction of SMN2 Splicing Defect

Hasane Ratni,[1] E. Pinard,[1] L. Green,[1] L. Mueller,[1] I. Gerlach,[1] F. Metzger,[1] M. Sivaramakrishnan,[1] K. S. Chen,[2] K. D. McCarthy,[2] S. V. Paushkin,[2] N. A. Naryshkin,[3] M. Weetall,[3] A. Dakka,[3] G. M. Karp,[3] J. Narasimhan,[3] X. Zhao,[3] H. Qi,[3] M. G. Woll,[3] G. Chen,[3] N. Zhang,[3] V. Gabbeta,[3] P. Vazirani,[3] A. Bhattacharyya,[3] B. Furia,[3] N. Risher,[3] J. Sheedy,[3] R. Kong,[3] J. Ma,[3] A. Turpoff,[3] S. Lee,[3] X. Zhang,[3] Y.-C. Moon,[3] E. M. Welch[3]

1) F. Hoffmann-La Roche Ltd., pRED, Pharma Research & Early Development, Grenzacherstrasse 124, 4070 Basel, Switzerland
2) SMA Foundation, 888 Seventh Avenue, Suite 400, New York, NY 10019, USA
3) PTC Therapeutics, 100 Corporate Ct, South Plainfield, NJ 07080, USA

Spinal muscular atrophy (SMA) is the leading genetic cause of mortality in infants and toddler and currently only palliative treatments are available. It is caused by the reduced expression of the survival of motor neuron (SMN) protein due to loss of functional SMN1 gene and alternative splicing of exon 7 in the SMN2 gene.

At the end of 2011, PTC Therapeutics, the SMA Foundation and F. Hoffmann-La Roche, Ltd entered into a unique three party collaboration to develop urgently a life changing treatment for children with SMA.

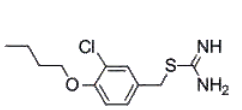
The strategy was to identify orally available novel small molecules that specifically modify SMN2 splicing in SMA patient-derived cells, increasing the production of full length SMN2 mRNA and consequently functional SMN protein production. Upon oral administration, the SMN protein level was restored in two mouse models of SMA, and subsequently, a dramatic increase in the life-span of delta 7 mice, a model of severe SMA, was achieved. This program is currently in clinical trials.

LE092 | Fragment-Based Discovery of BACE Inhibitors for Alzheimer's Disease

Andrew Stamford

Merck Research Laboratories, 126 E Lincoln Ave, Rahway, NJ 07065, USA

Alzheimer's disease (AD) is a devastating neurodegenerative disease for which there are no approved disease-modifying treatments. Although our understanding of the underlying disease pathogenesis is incomplete, accumulation of amyloid (Ab) peptides is widely considered to play a central role in the neurodegenerative process. The Ab peptides are produced by cleavage of the amyloid precursor protein (APP) by the aspartyl protease BACE1, followed by cleavage of the APP C-terminal fragment by g-secretase. Because accumulation of Ab peptides is implicated in AD pathogenesis, inhibition of BACE1 is a potential disease-modifying approach for the treatment of AD. However, the development of selective, brain-penetrant BACE1 inhibitors has proven to be a major challenge. To overcome this challenge, we have designed a novel series of iminoheterocyclic BACE1 inhibitors by application of fragment-based screening and X-ray crystallography-guided structure-based design. Fragment screening of BACE1 by HSQC NMR resulted in identification of low-affinity hits represented by **1**. Unique hydrogen-bonding interactions between the isothiourea of **1** and the catalytic dyad of BACE1 were revealed by X-ray crystallography, which led to the design of a unique BACE inhibitor scaffold represented by cyclic acylguanidines **2** and **3**. Optimization based on the key pharmacophore embodied by lead inhibitors **2** and **3** resulted in identification of potent, orally bioavailable and centrally active inhibitors exemplified by **4**. The property- and structure-driven evolution of this novel series of BACE1 inhibitors will be discussed, and an update on the status of the lead BACE1 inhibitor, MK-8931, currently in Phase 3 clinical trials for AD, will also be provided.



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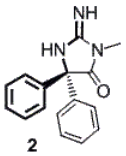

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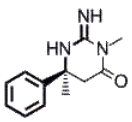

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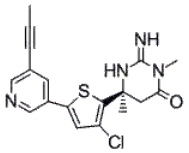

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LE093 | The Evolution of an In Vivo Efficacious BACE1 Inhibitor

Daniel Oehlrich,[1] Michiel Van Gool,[2] Juan Antonio Vega,[2] Francisca Delgado,[2] Oscar Delgado,[2] Andres Trabanco,[2] Gary Tresadern,[3] Frederik Rombouts,[1] Carolina Martinez-Lamenca,[1] Sven Van Brandt,[1] Michel Surkyn,[1] Michel De Cleyn,[1] Francois Bischoff,[1] Adriana I. Velter,[1] Chiara Zavattaro,[1] Yves Van Roosbroeck,[1] Frans Van Den Keybus,[1] Peter Buijnsters,[1] Ann Vos,[3] Gregor Macdonald,[1] Harrie Gijsen[1]

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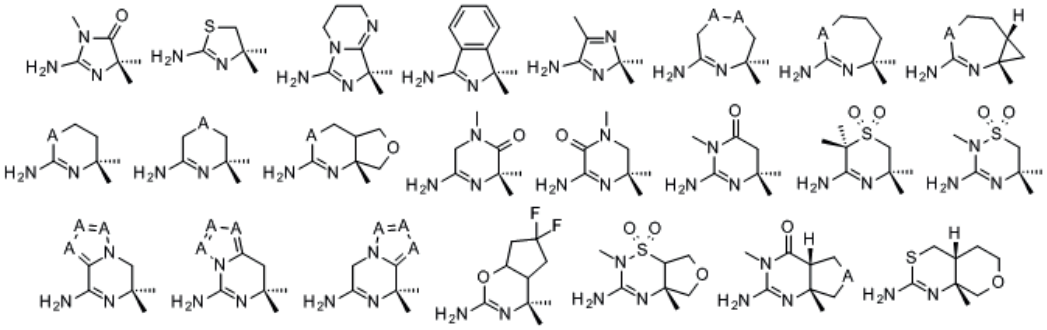
Alzheimer's disease (AD) is identified as a devastating disease and is characterized by the existence of two pathological features: amyloid plaques and neurofibrillary tangles. The pathology of these features has led to the development of the amyloid cascade hypothesis, which to date remains polemic as it has not been fully validated.

During the last two decades, many potent BACE1 inhibitors have been described but very few successfully display the desired balance of in vitro potency and the necessary PK properties/parameters to achieve in vivo efficacy. The identification of amidine- and guanidine-containing heterocycles was achieved via both HTS and fragment-based screening. Work around 5,5-disubstituted aminohydantoins by Schering-Plough and Wyeth in 2005 showed the formation of an ideal hydrogen-bonding network with the catalytic aspartyl dyad of BACE and highlighted the importance of the compact amidine-containing warheads with vectors that optimally distribute groups within the active site.

The need for an amidine-based functional group that shows high affinity for the catalytic dyad and as such has to be protonated in the target tissue within this class of BACE1 inhibitors has resulted in many difficulties for development, including Pgp, permeability and hERG. Chronologically, within the BACE field, we have seen the modulation of this basic center, maintaining the possibility to be protonated as required, in ever more inventive ways, while not infringing on the chemical space of other R&D investigations. The Figure shows the diversity of chemotypes that have been explored.



From this series of amidine-based BACE1 inhibitors, several compounds have now reached clinical trials. The results of these trials are likely to validate not only the amyloid hypothesis but also whether extended inhibition of BACE1 is therapeutically viable as a treatment of AD. This presentation will focus on the evolution of the amidine-based BACE1 inhibitor field concentrating on the control of Pka, Pgp, permeability and in vivo efficacy. This will be illustrated by our internal efforts in the development of a safe and efficacious BACE1 inhibitor.

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LE094 | CHF-6001, an Inhaled PDE4 Inhibitor for Asthma and COPD: Synthesis, In Vitro and In Vivo Characterization

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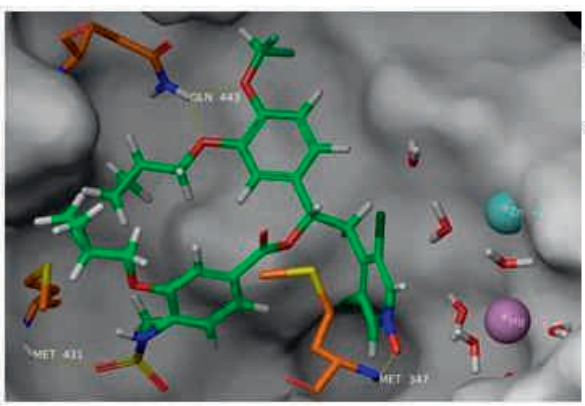
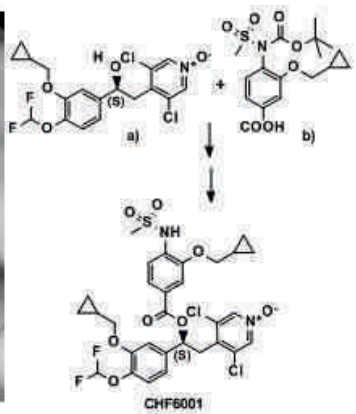
The phosphodiesterase 4 (PDE4) enzyme is highly expressed in inflammatory and immune cell types relevant for the pathogenesis of airway inflammatory disorders.[1] Indeed, PDE4 inhibition, resulting in an increase of intracellular cAMP concentration, exerts a broad range of anti-inflammatory effects. The recent approval of roflumilast as an oral drug for COPD treatment defines the standing of PDE4 as a therapeutic target in respiratory diseases. Since the clinical efficacy of oral PDE4 inhibitors is limited by target-associated systemic side effects such as nausea, diarrhea and headaches, the search for third-generation inhibitors endowed with a greater therapeutic window[2] is an area of great interest. Drug administration via inhalation is a strategy to achieve efficacy directly to lung tissue with minimal unwanted systemic effects. Here we report the synthesis and the in vitro and in vivo pharmacological characterization of CHF6001, purposely designed to reach high potency vs PDE4 enzyme and to be suitable for inhaled administration.

Our medicinal chemistry strategy led to the discovery of compounds endowed with an improved in vitro potency and in vitro ADME properties suitable for inhaled administration.[3] CHF6001 proved to be the most interesting compound displaying the best combination of high potency in cell-free and cell-based assays, high stability in lung tissue, low permeability, high PPB, and, upon topical administration in relevant animal models, resulted in highly significant and long-lasting anti-inflammatory effects. CHF6001 was obtained as a crystalline solid by a double-branched convergent route in which the key intermediates a) and b) are connected in the penultimate step of the synthesis. A docking study of CHF6001 in the PDE4B catalytic site showed an extended binding interaction with the hydrophobic region, the metal region and the solvent exposed region.





CHF6001 is equally potent to GSK256066 and 6-fold more potent than roflumilast in inhibiting PDE4 enzymatic activity (IC_{50}=0.026 nM). CHF6001 inhibits recombinant human PDE4 isoforms A–D with equal potency while shows >20,000-fold selectivity in comparison with other PDE isoenzymes. CHF6001 displays remarkable anti-inflammatory potencies (sub-nanomolar IC_{50} values) in several cell-based assays: hPBMC, THP-1 macrophagic cells, $CD4^+$ T cells, eosinophils and macrophages. When administered intratracheally to rats as a micronized dry powder, CHF6001 exerts an extremely potent anti-inflammatory activity (ED_{50}=0.03 μmol kg^{-1}) in Ovalbumin-mediated inflammatory responses. In an 11-day mouse model of tobacco smoke-induced inflammation, CHF-6001 significantly inhibits neutrophil recruitment both as prophylactic and interventional treatment, at the lowest doses administered (0.15 and 0.045 μmol kg^{-1} intranasally, respectively).

In conclusion CHF6001, showing the potential to be an effective topical agent for conditions associated with pulmonary inflammation, may represent a novel therapeutic option for the treatment of asthma and COPD.

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LE095 | The Discovery of AZD5069: A CXCR2 Antagonist in Phase II Clinical Trials for the Treatment of Asthma

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Inflammation driven by neutrophils is implicated in lung damage in inadequately controlled persistent asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis. Neutrophil recruitment is predominantly driven by activation of CXC chemokine receptor 2 (CXCR2) and blockade of this receptor inhibits the binding of IL-8 and GRO-alpha, and consequently neutrophilic migration to the lung. Antagonism of CXCR2 therefore presents an attractive strategy for treatment of these inflammatory diseases. We have identified AZD5069 as an oral, selective and reversible CXCR2 antagonist that targets neutrophil-driven inflammation. This compound is in clinical development at AstraZeneca to evaluate its effect on the frequency of severe exacerbations, asthma symptoms and health-related quality of life. The discovery and optimization of lead compounds, together with the structure and profile of AZD5069, will be presented.

LE096 | Discovery of BAY 85-8501, a Novel and Highly Potent Induced-Fit Binder of Human Neutrophil Elastase for Pulmonary Diseases

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Human neutrophil elastase (hNE) is a key enzyme for matrix degradation. In inflammatory diseases, high HNE activity is observed. HNE has been discussed as a target in pulmonary diseases such as bronchiectasis, chronic obstructive pulmonary disease (COPD), acute lung injury (ALI), acute respiratory distress syndrome and pulmonary hypertension (PH). Elastase inhibitors could re-establish the protease anti-protease balance in these diseases.

The first potent elastase inhibitors described were biologicals. The first small-molecule inhibitors were reactive acylators or transition-state mimetics. In general, selectivity is a high hurdle for serine protease inhibitors.

Starting from a sub-micromolar quinoline inhibitor that was found in a high-throughput screen, various chemical core modifications finally led to dihydropyrimidinones as a novel lead structure class with frozen bioactive conformation. Further chemical optimization of physicochemical parameters and metabolic stability yielded orally active compounds with favorable pharmacokinetics in rodents and dogs. Finally, a novel potent and selective class of small molecule hNE inhibitors was discovered. BAY 85-8501 showed picomolar in vitro potency versus hNE and high selectivity towards 21 serine proteases. These favorable characteristics are based on an induced-fit binding mode, allowing for tight interactions with the S2 and the S1 pocket. BAY 85-8501 showed in vivo activity in rodent animal models related to PH and ALI.

LE097 | Discovery of TD-5959 (Batefenterol/GSK-961081): A First-in-Class Dual Pharmacology Multivalent Muscarinic Antagonist and Beta-2 Agonist (MABA) for the Treatment of COPD

Adam D. Hughes, Yan Chen, Sharath S. Hegde, Jeffrey R. Jasper, Sarah Jaw-Tsai, Tae-Weon Lee, Alexander McNamara, Mathai Mammen, M. Teresa Pulido-Rios, Tod Steinfeld

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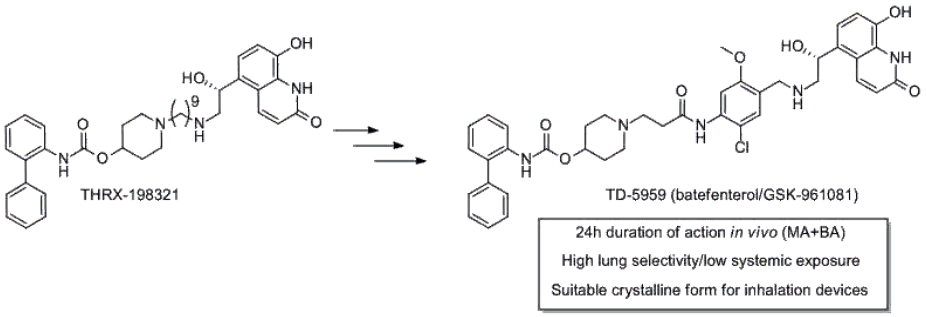
Through application of our multivalent approach to drug discovery we previously reported the first discovery of dual pharmacology MABA bronchodilators, exemplified by THRX-198321. Herein, we describe the subsequent lead optimization of both muscarinic antagonist and β_2 agonist activities, through modification of the linker motif, to achieve 24h duration of action in a guinea pig bronchoprotection model. Concomitantly, we targeted high lung selectivities, low systemic exposures and identified crystalline forms suitable for inhalation devices. This article culminates with the discovery of our first clinical candidate TD-5959 (batefenterol/GSK-961081). In a Phase 2b trial, batefenterol produced statistical and clinically significant differences compared to placebo and numerically greater improvements in the primary endpoint of trough FEV_1 compared to salmeterol after four weeks of dosing in patients with moderate to severe COPD.



LE098 | Identifying Selective Inhibitors of FGFR4 Kinase

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The fibroblast growth factor receptor (FGFR) family of receptor tyrosine kinases consists of four family members, FGFR1–4, which bind to 22 different FGF ligands. These binding interactions result in receptor homodimerisation and autophosphorylation, recruitment of cytosolic adaptors such as FRS2 and initiation of multiple signalling pathways (e.g., MAPK and PKB/Akt pathways). FGFR4 is overexpressed in several types of human tumours including colon, liver, breast, neuroendocrine and pancreatic carcinoma and a mutation in the transmembrane domain of the FGFR4 gene (FGFR4 G388R) has been associated with poor prognosis in several malignancies. FGF19 binds uniquely to FGFR4 and researchers have shown that targeting FGF19-FGFR4 signalling with an anti-FGF19 monoclonal antibody results in tumour growth inhibition in colon cancer xenograft and FGF19 transgenic hepatocellular carcinoma models.

Several potent and selective small molecule inhibitors of FGFR have been reported over the last decade; however, many of the compounds appear to be significantly more potent against FGFR1–3 than FGFR4. The reduced potency of such agents against FGFR4 would necessitate the use of high doses in order to effectively treat FGF19-FGFR4-driven tumours and such doses might result in complications associated with either the potent inhibition of FGFR1–3 or through activity against other kinases. Herein we report our efforts to identify selective small molecule inhibitors of FGFR4 using structural knowledge of the highly conserved FGFR1–4 ATP binding sites to guide our efforts and culminating in compounds demonstrating in vivo activity. Strategies aimed at delivering reversible and irreversible inhibitors will be discussed.

LE099 | Abstract unavailable at the time of printing

LE100 | Non-clinical Characterization and Clinical Translation of LY2811376 and LY2886721

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1) Eli Lilly and Company
2) Takeda Pharmaceuticals
3) PAREXEL International
4) Novartis
5) UCB Pharma
6) Constellation Pharma

Cerebral deposition of amyloid-β peptide (Aβ) is critical in Alzheimer's disease (AD) pathogenesis. Owing to its role in the generation of Aβ, the BACE1 enzyme continues to be a prime target for designing drugs to prevent or treat AD; however, BACE1 has proven to be an exceedingly challenging target for drug discovery. In 2010, we demonstrated for the first time that LY2811376, a small molecule BACE1 inhibitor discovered using a fragment-based approach, could produce profound Aβ-lowering effects in humans. We subsequently advanced a more potent and selective BACE1 inhibitor, LY2886721, which ultimately progressed into phase II clinical development. This presentation will describe the preclinical and clinical PK/PD characterization of LY2811376 and LY2886721, including biomarker translation from beagle dogs to healthy volunteers and patients with Alzheimer's disease.

LE101 | Application of Translational PKPD Modeling to Predict the Effective Dose of Siponimod A S1P Antagonist in Human Based on Preclinical Data

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Siponimod is a next-generation selective sphingosine 1-phosphate (S1P)-1 and -5 receptor modulator administered once daily orally. Siponimod reduces lymphocyte infiltration into the CNS and may have direct CNS effects. In relapsing multiple sclerosis, S1P receptor modulation reduces accumulation of neurological impairment and slows progression of brain atrophy. During the development of siponimod the objective was to anticipate the minimal active biological effective level (MABEL) in human in order to start the first in man study. Another objective was to anticipate the clinical desired dose (CDD: a dose that reduces the absolute peripheral lymphocyte count). Translational PKPD modeling was used to predict pharmacokinetics, pharmacodynamics, MABEL and CDD of siponimod. A physiological-based pharmacokinetics/pharmacodynamics was used to predict the relationship between pharmacokinetics and pharmacodynamic in man. The input parameters of the model were drug related (e.g. log*D*, pK_a, BP, Cl, V_{ss} and species specific), (e.g., *Q*, *V*, tissue composition). Clearance was scaled to human based on allometry using preclinical data. One basic assumption was that the drug potency (IC_{50}) of Siponimod was similar between monkey and human. Despite known interspecies differences at the level of S1P receptors expression, anticipation of PK, PD, MABEL and CDD were predicted successfully.

LE102 | Biomarkers after Bacteriochlorin-Based Photodynamic Therapy against Cancer

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Introduction: Photodynamic therapy (PDT) employs the combination of nontoxic photosensitizers and harmless visible or near infrared light to generate reactive oxygen species (ROS) resulting in cell death by apoptosis or necrosis and eventually the stimulation of an immunoresponse. Depending on the treatment protocol, PDT has a strong influence on tumor vasculature. The aim of this study was to characterize biomarkers to predict efficiency of anticancer therapy.

Materials and Methods: Tumor models: S91/I3 Cloudman melanoma and Levis Lung carcinoma tumors were grown in the legs of DBA/2 and C57BL/6J mice. PDT: When a tumor reached a mean diameter of 3–5 mm, 2–4 mg/kg BW of F_2 BMet[1,2] were administered intravenously into mice, and 15 min later (VTP) or 72 h later (CTP) tumors were treated with 70–140 J/cm^2 of light at λ=750 nm. EPR oxymetry: LiPc (solid-state spin probe) was implanted into the tumor to estimate local changes in tissue oxygenation after PDT at several time points before and after PDT. PW/Doppler-USG: a VEVO 2100 ultrasonograph was used to measure structure and function of vasculature before, and 15 min, 3 h after treatment, and then every 24 h for 6–16 following days. IHC: allowed us to visualize neutrophils, macrophages, vasculature, hypoxia and morphology of tumors at certain time points after treatment (15 min and 3, 24, 48, 96, 144 h). Western Blot (WB) and ELISA assays: 24 h after PDT, the tumor and plasma samples were collected from mice. After tissue homogenization, the protein level was calculated in each sample. The level of HO-1, MMP2, MMP9, Cas-3, Cas-9, NFkB, COX2, VEGF, TNF and wide range of cytokines and chemokines was investigated.

Results and Discussion: PDT induced radical changes in vasculature structure and function depending on treatment protocol. VTP caused a decrease of function of vasculature already a few minutes after therapy (based on USG, EPR). After CTP, a significant decrease in vessel density was seen at 24 h and was followed by an increase in the next days, to a much higher level than in the case of VTP. The pO_2 changes after treatment can be valued biomarker. The strong immune response was observed after PDT, with differences between treatment protocols. Detailed analysis of ELISA and WB assays allow us to select for further analysis a few proteins as promising biomarkers.

Conclusions: pO_2 in the tumor tissue and some proteins (e.g., IL-6) can be selected as new, promising, post-therapeutic biomarkers of bacteriochlorin-PDT.

Acknowledgements:

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