Late Abstracts

PL001 | High-Resolution Mass Spectrometry in Clinical Proteomics

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Mass spectrometry (MS)-based proteomics, particularly in a quantitative and high-resolution format, has become a very powerful technology to study gene expression at it 'end point'—the level of proteins. In this talk, I will give an overview of technological developments and established and new application areas. Recent improvements in the shot-gun proteomic workflow, especially in the areas of sample preparation, chromatography, mass spectrometry and computational analysis now allow near complete quantification of the yeast proteome in just a few hours—a task that previously took weeks of measurement time.

One exciting new capability relies on 'single-run' or 'single-shot' proteomics, in which a relatively long HPLC column is coupled to a high-resolution, bench-top mass spectrometer with very high sequencing speed. It uses minimal amounts of sample and allows measurement of many states of the proteome while remaining unbiased as no subgroup of the proteome needs to be selected for analysis. We use this approach routinely for the comprehensive analysis of model proteome, in-depth characterization of human cancer cell lines and increasingly complete analysis of mammalian tissues.

Furthermore, MS-based proteomics can analyze post-translational modifications on a very large scale—for example, more than 50,000 phosphorylation sites can readily be detected in a cell line and modifications such as ubiquitylation and acetylation are also readily accessible. Quantitative proteomics can also very accurately detect specific protein interactions, for instance with other proteins, with modified peptides or with small molecules. In this format, protein quantification is applied to distinguish background binders from true binders. By quantification of binders to bait molecules vs. a control bait, the need for stringent washes is reduced and transient binders can still be detected. Applications of this generic workflow include interactions with specific DNA elements in the genome (such as GWAS derived SNPs or QTLs), RNA structures, post-translational modifications of proteins as well as interactions of small molecules with proteins.

Proteomics is now capable of relatively rapid characterization of human tumor samples. One of the clinically important challenges in oncology is the classification of patients into subgroups with different risk profiles and treatment modalities. Using SILAC-based or label-free proteomics, we have successfully distinguished the ABC and GBC subtypes of Germinal center B-cell like diffuse large B-cell lymphoma (DLBCLs) in cell lines derived from patients. We are now studying cohorts of intermediate sizes in breast, ovarian and prostate cancer. We have also revisited the analysis of body fluids, such as lung lavage and plasma, using the latest technological advances and I will discuss where these efforts stand at the moment.

PL004 | Innovation through Chemistry at the Interfaces of the Scientific Disciplines

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The central role and multi-faceted impact of chemistry in drug discovery will be discussed based on projects from our research efforts.

C022 | Open Innovation Antitubercular Drug Discovery between Industry and Academia: The OpenMedChem Project

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Recently, GlaxoSmithKline (GSK) has completed an antimycobacterial phenotypic high-throughput screening (HTS) campaign against *Mycobacterium bovis* BCG with hit confirmation in the *M. tuberculosis* H37Rv strain. In an unprecedented endeavor to stimulate open-source drug discovery, the results of this campaign have been made publicly available. They provide many potential new starting points for synthetic lead-generation approaches. Following the Open Innovation strategy of the company, the OpenMedChem project has been established within the framework of Marie Curie Actions (FP7-PEOPLE-2012-ITN). It aims at a true collaboration between academia (University of Antwerp, Belgium) and industry (GSK I+D, Madrid, Spain).

The scientific focus of this project is the design and synthesis of novel analogues of the most promising hit compounds, identified in the GSK screening campaign. In particular, the HTS has delivered several scaffold families that were selected after passing multiple antimicrobial activity and drug-like property screens. These families can be considered as high quality lead precursors and thus fit for in depth chemical and biological investigation. Detailed structure–activity relationship (SAR) and in vitro biological studies will be performed accompanied by thorough antimycobacterial profiling and gross mode of action studies. Specifically, the activity will be evaluated using standard MIC assays, complemented with novel cidality, intracellular activity assays as well as single-cell imaging-based approaches.

I015 | 7β,15α-Dihydroxy Lupeol Esters Isolated from Nigerian Mistletoe Parasitic on *Kola acuminata* Exhibits Potent Inhibitory Activities against IL-8 Expression and Mitogenic Effect on C57BL/6 Mice Splenocytes and CD69 Molecule In Vitro

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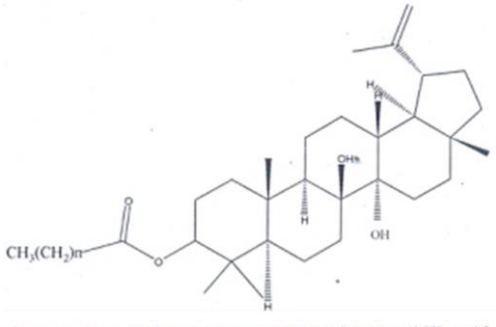
In our continued efforts to validate further the established immunomodulatory and anti-inflammatory activities of the Nigerian mistletoes, palmitate (**I**), stearate (**II**) and eicasanoate (**III**) esters of 7β,15α-dihydroxylupeol were isolated in significant yield (≈6.6 %) from potently immunostimulatory *n*-hexane fraction of the extract of mistletoe parasitic on *Kola acuminata* and characterized. Compound **I** was evaluated for its effect on IL-8 expression using RT-PCR assay while the effects on C57BL/6 mice splenocytes, and the CD69 molecule were measured by flow cytometry. At 1 µg/mL and 5 µg/mL concentrations of the compound, respectively, IL-8 expression was inhibited by 98.34 ± 0.17 % and completely abolished compared to positive standard, *Juzen-taito-to* and control. Higher concentrations ≥10 µg/mL competitively blocked all IL-8 receptors. Similarly, compound **I** at 100 µg/mL concentration enhanced C57BL/6 mice splenocytes and the CD69 molecule proliferation by 86.98 ± 0.06 % and 24.44 ± 2.58, respectively, compared to 220.71 ± 0.11 % and 34.01 ± 0.32 for LPS and Con A. Taken together; these data suggest significant immunogenic and potent anti-inflammatory activities of 7β,15α-dihydroxylupeol palmitate (**I**) isolated from Nigerian mistletoes parasitic on *Kola acuminata.*



Compound I, n=12; Compound II, n=14 and Compound III, n=16

Acknowledgements:

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L040 | Medicinal Chemistry Profile of a New Trypanomicide Prototype: LASSBio-1064

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The Kinetoplastids are a group of flagellate protists responsible for some diseases in humans and other animals. Among them, the members of Trypanosomatidae family, exclusively, parasitize humans. Discovered in 1909 by Carlos Chagas, Chagas' disease is a disease caused by the species *Trypanosoma cruzi*, whose treatment is based on the use of only two drugs, nifurtimox and benzinidazol, both with various adverse effects and ineffective in the treatment of chronic states of the disease.[1] This summary describes the medicinal chemical profile of a new trypanomicide prototype (LASSBio-1064) discovered at LASSBio®-UFRJ (Laboratório de Avaliação e Síntese de Substâncias Bioativas).

The *N*-acylhydrazones (NAH) are considered privileged structures being present in the molecular framework of several anti-inflammatory, analgesic, antiparasitic prototypes, among others.[2] Thus, a screening was carried out to evaluate the in vitro activity of a series of trypanomicide NAH derivatives, rationally selected from the chemolibrary of LASSBio®. This screening highlighted LASSBio-1064, which presented an important activity profile against *T. cruzi* epimastigotes (CLBrener strain) in doses ≤ 1 μM. This new trypanomicide ligand was assayed in vivo, by oral administration, in order to assess its anti-chagasic potential using a murine chagasic acute infection model and employing benznidazole as standard drug (Table 1).

Table 1: Effect of the treatment with LASSBio-1064 in mice experimentally infected with T. cruzi (acute phase).

Days post-infection	Decrease in Number of parasites (%)		
	Control	Benznidazole (50 mg)	LASSBio-1064 (5 mg)
60	0	58	99,9

In this assay was possible to identify that LASSBio-1064 presented anti-chagasic activity in dose 10 times smaller than the standard benznidazole. In order to establish the safety profile of LASSBio-1064, this compound was evaluated in tests of cytotoxicity, genotoxicity and mutagenicity using human lymphocytes. The results obtained revealed the absence of genotoxicity and mutagenic profile of LASSBio-1064. Otherwise, a dose and time dependent cytotoxic profile was observed.

In conclusion, a new trypanomicide prototype was discovered: LASSBio-1064. It possesses oral anti-chagasic activity at a dose 10 times lower than standard benznidazole. Later studies showed that this prototype has a cytotoxic effect with IC_{50} = 2.69 μg/mL, and is devoid of genotoxicity and mutagenicity.

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M022 | New Targets against Neurodegenerative Diseases; Dual Action against Oxidative Stress

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As a result of the aging population, neurodegenerative diseases, such as Alzheimer's Disease (AD), Parkinson's Disease (PD) or ischemic stroke, afflict an ever-increasing number of people. Although their pathological pathways are not yet fully understood, oxidative stress and mitochondrial dysfunction are thought to play a key role on their onset and development.[1] Therefore, increasing efforts are devoted to the development of new therapeutic agents targeting these alterations.

Based on these observations, we focused our efforts on the design, synthesis and biological evaluation of a new compound directed to up-regulate the antioxidant response element present in the neurons, by liberating its regulator, the nuclear erythroid-related factor 2 (Nrf2). Thus, we have developed a new multi-target directed ligand (MTDL) that exerts a dual mechanism of action, being able to induce the liberation and nuclear translocation of the Nrf2 factor and, also, to act as a potent free radical scavenger.

Its pharmacological characterization included the study of its neuroprotective profile against two different "in vitro" models of oxidative stress; rotenone/oligomycin A, and oxygen-glucose-deprivation (OGD) in hippocampal slices, showing an interesting neuroprotectant profile. The study of its mechanism of action has revealed that this MTDL exerts its neuroprotection by i) reduction of free radicals production, ii) Nrf2-ARE induction, iii) effects on GSH cell content, and iv) overexpression of phase II neuroprotective enzymes such as HO-1.

This compound, developed as "proof of concept" of a new family of MTDLs directed to non-conventional targets for the treatment of neurodegenerative diseases, may lead to a new family of compounds with improved activity.

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O025 | Crowdsourcing Chemical Scaffolds and Biological Targets—A Large-Scale, Controlled Experiment

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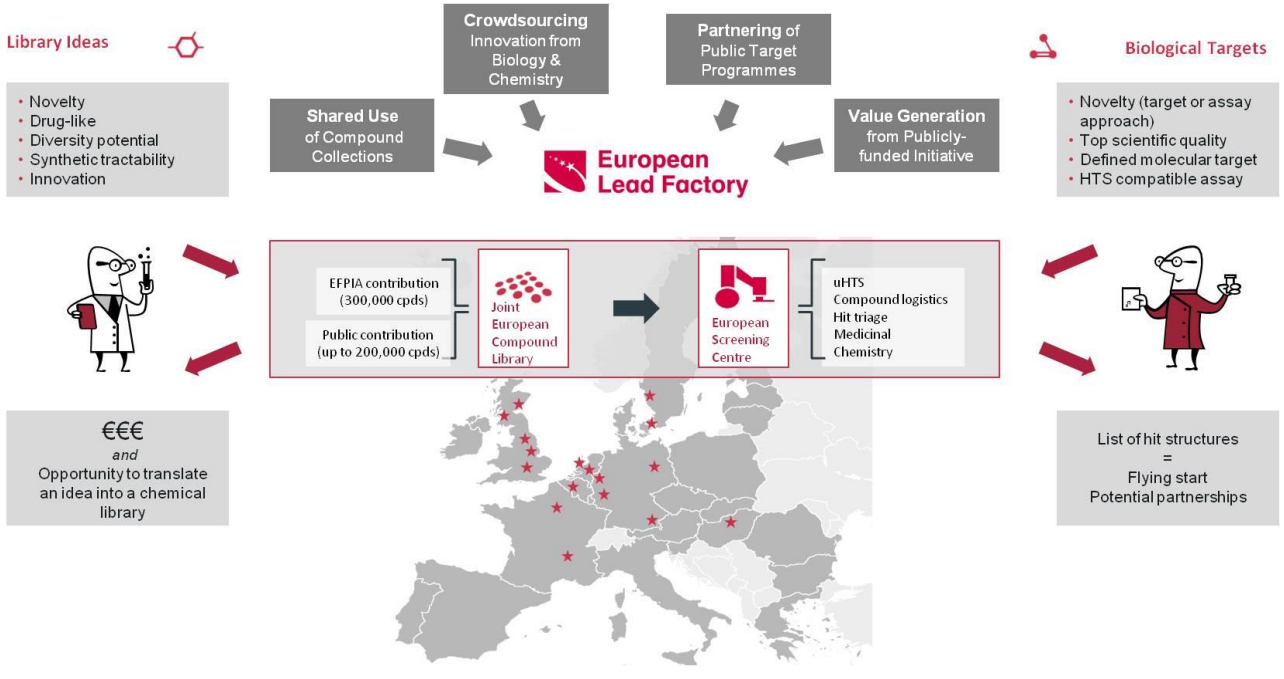
The EU Lead Factory is the first pharmaceutical and life sciences partnership of its kind. It has been designed to create unrivalled opportunities for the discovery of new drug lead molecules. Academics and SMEs now have access to an 'industry-like' discovery platform. Scientists with innovative biology target and chemistry scaffold owners are welcome to participate in the EU Lead Factory.



Seven large pharmaceutical companies have joined forces and contributed proprietary compounds to the core Joint European Compound Library (JECL). Targets from public partners are screened at the top-modern, industry-standard European Screening Centre (ESC) at no upfront cost, as funding is provided by the IMI. Further exploitation of generated hits is subject to a number of conditions.

This unique large-scale experiment, combining open innovation, crowdsourcing and several established pharmaceutical companies, is now producing its first scientific results.

Acknowledgements:

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R064 | Rational Design of Novel Catalytic Human DNA Topoisomerase IIα Inhibitors

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Human DNA topoisomerases II are an important and validated targets for the development of novel anticancer agents.[1] Inhibitors targeting human DNA Topoisomerase IIα generally fall into two groups that differ in their mechanism of action. An established group of poisons which stabilize the covalent cleavable complex and convert this enzyme into a cellular toxin, and an emerging group of catalytic inhibitors that act by interfering with a single step of the topo II catalytic cycle.[2,3]

Based on the available structural information about the binding mode of the AMP-PNP molecule to human topo IIα,[4] we outlined a two-stage virtual screening campaign combining structure-based and ligand-based pharmacophore models with molecular docking calculations.[5] This screening led to the discovery of 1*H*-pyrazolo[3,4]pyrimidine-4,6-diamine inhibitors of DNA topoisomerase IIα in lower micromolar range and 4-amino-6-(phenylamino)-1,3,5-triazines in micromolar range. The binding to human DNA Topoisomerase IIα ATPase domain and formation of the ligand-enzyme complex was further substantiated by using SPR measurements and MD simulations. Selected compounds from all these classes also showed promising anticancer activities in hepatocellular carcinoma cell line (HepG2) and breast cancer cell line (MCF-7). Discovered compounds represent promising starting points for further hit to lead development in the discovery of novel anticancer agents.

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R065 | 2',4'-Dihydroxy-3,4,5-trimethoxychalcone Acts as an Antimitotic Agent and Induces Mitotic Catastrophe in MCF-7 Breast Cancer Cells

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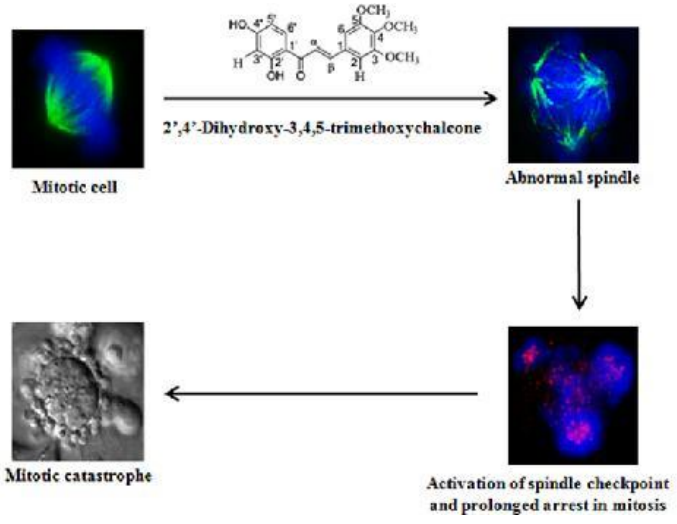
2',4'-Dihydroxy-3,4,5-trimethoxychalcone was previously synthesized and evaluated as a growth inhibitory agent in the three human cancer cell lines MCF-7, NCI-H460 and A375-C5.[1] Here, we show the results concerning the investigation of the mechanism of the anti-growth action on human breast adenocarcinoma MCF-7 cells. We found that the compound induced multipolar and monopolar spindles, hence perturbing mitotic spindle assembly, suggesting an anti-microtubule activity. Compound-treated cells arrested at metaphase with misaligned chromosomes and triggered the spindle assembly checkpoint, as evaluated by kinetochore accumulation of Mad2, Bub1 and BubR1 proteins, indicating an anti-mitotic activity. Time-lapse analysis revealed that the compound sustained a prolonged arrest in mitosis, followed by massive apoptotic cell death as confirmed by TUNEL assay. Collectively, our data indicate that 2',4'-dihydroxy-3,4,5-trimethoxychalcone acts as an anti-mitotic agent that exerts its growth inhibitory activity on MCF-7 cells by inducing mitotic catastrophe.[2]



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V054 | The INPHARMA-Based Design of Cdk-2 Inhibitors

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The major impediment in structure-based drug design is insufficient information about the interactions between small molecules and their target protein. Although crystallographic and protein-based NMR methods provide superb input to the protein–ligand interaction, the inability to obtain crystals for many proteins of interest, in parallel with a 50 kDa cut off for standard NMR techniques, highlights the need for alternative approaches. In this study, we use the INPHARMA method (Interligand NOEs for Pharmacophore Mapping), which provides access to the relative binding mode of two low-affinity ligands binding competitively to a common target.[1] This method identifies the correct ligand binding pose through scoring of computationally generated docking poses with experimentally obtained ligand-based NOE data. The ligand binding poses obtained through INPHARMA can be further used for the generation of a 3D pharmacophore.

In this project, we take advantage of the method and generate ligand- and structure-based 3D pharmacophores of Cdk-2 binders; these pharmacophores were employed in the virtual screening of the ZINC database. We show that INPHARMA offers a rapid alternative to obtaining high-resolution information on protein–ligand interactions, without the need for extensive optimization of crystallographic conditions or the assignment of protein resonances for NMR studies. The method is especially relevant to the design of new leads for proteins that do not crystallize or that are too big to be studied by NMR.

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W014 | Intramolecular Acyl Cyclisation (IAC) as a Synthetic Strategy for the Synthesis of Erythrina Alkaloid Derivatives

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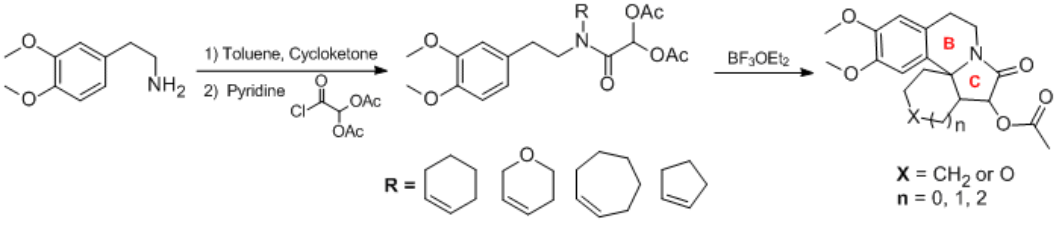
The family of compounds known as the erythina alkaloids are isolated from *Erythrina* plants found in tropical and sub-tropical regions.[1] These alkaloids have attracted considerable attention due to their anticonvulsant, antidepressant, analgesic sedative and hypnotic effects.[2] Erythrina alkaloids have a characteristic tetracyclic spironamide structure, and in the last decades, a number of synthetic approaches have been adopted to obtain this framework, including radical and Pummerer cyclisations, intramolecular condensation and Diels–Alder reactions.[3,4] In an effort to develop an alternative route for the synthesis of this class of alkaloids, we focused on a Lewis acid catalysed intramolecular acylal cyclisation (IAC) which proceeds via formation of an iminium ion. This new methodology is efficient, reliable, and enables the formation of the B and C rings in a one pot reaction over two steps with good yields (see Figure). The results of our investigations to this family will be described.



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Y071 | Extended Structure–Activity Relationship and Pharmacokinetic Investigation of (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Inhibitors of Fibroblast Activation Protein (FAP)

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Fibroblast activation protein (FAP) is a serine protease related to dipeptidyl peptidase IV (DPP IV). It has been convincingly linked to multiple disease states that involve remodeling of the extracellular matrix. FAP inhibition is investigated as a therapeutic option for several of these diseases, with most attention so far devoted to oncology applications. We previously discovered the *N*-4-quinolinoyl-Gly-(2*S*)-cyanoPro scaffold as a possible entry to highly potent and selective FAP inhibitors. In the present study, we explore in detail the structure–activity relationship around this core scaffold. We report extensively optimised compounds that display low nanomolar inhibitory potency as well as high selectivity against the related dipeptidyl peptidases (DPPs) DPP IV, DPP9, DPP II and prolyl oligopeptidase (PREP). The log*D* values, plasma stabilities, and microsomal stabilities of selected compounds were found to be highly satisfactory. Pharmacokinetic evaluation in mice of selected inhibitors demonstrated high oral bioavailability, plasma half-life and the potential to selectively and completely inhibit FAP in vivo.