First Annual Meeting

BOOK OF THE ABSTRACTS

COST ACTION CA15135

Multi-target paradigm for innovative ligand identification in the drug discovery process (MuTaLig)

Università della Svizzera Italiana – USI – Lugano (CH) – July 21, 22 2016

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INTRODUCTION

The MuTaLig COST Action aims to put together highly-qualified research teams working in several disciplines related to Medicinal Chemistry. The joining topic is the emergent multi-target issue, nowadays playing a central role in drug discovery. Its extremely multidisciplinary character is a good guarantee of strong interaction among all COST Action participants. From the starting five co-proposers the parties of our COST Action dramatically increased up to thirty nations.

The 1st annual meeting represents the first occasion of knowledge and exchange information among the research teams. The meeting is organized in plenary, oral and poster communications. Speakers will come from most of the joining countries and will include young scientists according to the gender balance. Qualified talks will be given also from private companies. The research competencies of the network span around medicinal chemistry, from synthetic chemistry, natural products and biophysics to theoretical chemistry, molecular modelling and biological screening. The two full days will be completed by a Management Committee meeting and a final round table.

As Chair of this COST Action I want to express my gratitude especially to the local organizers (Prof. Vittorio Limongelli and Dr. Eugenio Gaudio), to the Grant Holder from University of Porto (Prof. Fernanda Borges and Dr. Joana Maria Neves Moreira Abrantes) and to the COST Association (Dr. Lucia Forzi, Science Officer and Dr. Svetlana Voinova, Administrative Officer) for their efforts in the meeting organization.

I wish a fruitful and stimulating meeting to all participants!

Stefano Alcaro

Università Magna Græcia di Catanzaro (Italy)

Chair of CA15135 COST Action

PROGRAM

Thursday July 21st 2016

8.30 Registration

9.00 **Opening Introduction to the MuTaLig COST Action**
Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)

Session I
moderator Danijel KIKELJ (WG1 leader)
University of Ljubljana (Slovenia)

9.10 O1 **The first molecule interacting with a host protein for the inhibition of multiple viruses**
Maurizio BOTTA (CA15135 Industrial coordinator) - University of Siena (Italy)

9.30 O2 **Development of Ribonuclease H/DNA polymerase HIV-1 RT dual inhibitors**
Simona DISTINTO - University of Cagliari (Italy)

9.50 O3 **Multiple Targets Selection for Design of Inhibitors of Ebola Virus Infection**
Črtomir PODLIPNIK - University of Ljubljana (Slovenia)

10.10 O4 **Biofilm targets for the development of novel therapeutics**
Manuel SIMÕES - University of Porto (Portugal)

10.30 O5 **Design, synthesis and biological activity of novel dual ATP-competitive inhibitors of DNA gyrase and topoisomerase IV**
Tihomir TOMAŠIČ - University of Ljubljana (Slovenia)

10.50 *Coffee break*

Session II
moderator Christa MÜLLER (CA15135 IP coordinator) University of Bonn (Germany)

11.20 O6 **Standard and advanced simulations in drug design**
Vittorio LIMONGELLI – Università della Svizzera Italiana, Lugano (Switzerland)

11.40 O7 **3D-Chemical Feature Based Pharmacophores: Essential Tools for Early Drug Discovery Research**
Sharon BRYANT (WG3 leader) - Inte:Ligand GmbH, Vienna (Austria)

12.00 O8 **Let's start from the very beginning: The virtual screening work flow**
Hanoch SENDEROWITZ (WG4 leader) - Bar-Ilan University, Ramat-Gan (Israel)

12.20 O9 **Let's develop together an exchange virtual compounds computational platform**
Francesco ORTUSO - Università "Magna Græcia" di Catanzaro (Italy)

12.40 O10 **Discovering and designing new drugs considering toxicity and safety from the early stages incorporating multiple targets and anti-targets**
Alfonso GARCÍA-SOSA - University of Tartu (Estonia)

13.00 *Lunch*

Session III
moderator Maurizio BOTTA (MuTaLig Industrial coordinator) University of Siena (Italy)

14.10 PL1 **Patents: applications in the pharmaceutical field**
Marco ZARDI - Zardi & Co. Attorneys, Lugano (Switzerland)

14.50 O11 **Development of dual- and multi-target drugs for the treatment of neurodegenerative diseases**
Christa MÜLLER (CA15135 IP coordinator) - University of Bonn (Germany)

15.10 O12 **Development of dual target adenosine A1/A2A receptors antagonists among annelated xanthine derivatives**
Katarzyna KIEĆ-KONONOWICZ - Jagiellonian University, Cracow (Poland)

15.30 O13 **Antimicrobial activity of 1,8-naphthalimide metal complexes**
Ivo GRABCHEV - University of Sofia (Bulgaria)

15.50 O14 **Inhibitors of aldose reductase with multi-target properties**
Magdalena MAJEKOVA Slovak Academy of Sciences, Bratislava (Slovakia)

16.10 *Coffee break*

Session IV
moderator Sharon BRYANT (WG3 leader)
Inte:Ligand GmbH, Vienna (Austria)

16.40 O15 **Mitochondrial targets for multi-target ligand design**
Rona RAMSAY - University of St Andrews (UK)

17.00 O16 **Hydroxycinnamic acid as a valid scaffold for the development of multitarget ligands for CNS disorders**
Tiago SILVA - University of Porto (Portugal)

17.20 O17 **3D-QSAR and design of dopamine D3 receptor ligands**
Slavica FILIPIC - University of Belgrade (Serbia)

17.40 O18 **Multi-Objective Optimization in Drug Design**
Abraham YOSIPOF - Peres Academic Center, Rehovot (Israel)

20.20 *Social Dinner at Lanchetta Restaurant,*
Via Castagnola 16, Lugano

Friday July 22nd 2016

Session V
moderator Hanoch SENDEROWITZ (WG4 leader)
Bar-Ilan University, Ramat-Gan (Israel)

9.00 PL2 **PI3K inhibition (one Dalton apart)**
Doriano FABBRO (CSO, Piqur Therapeutics), Basel (Switzerland)

9.40 O19 **The BET Bromodomain inhibitor OTX015 affects pathogenetic pathways in preclinical B-cell tumor models**
and synergizes with targeted drugs
Eugenio GAUDIO (WG2 leader) Oncology Research Institute, Bellinzona (Switzerland)

10.00 O20 **Isoellipticine: targeting cell proliferation by a structured approach**
Florence McCARTHY - University College Cork (Ireland)

10.20 O21 **Identification of new multi-targeted molecules, designed as Neuropilin antagonists, with potent anti-angiogenic and anti-tumour activity in vivo**
Luc DEMANGE - CNRS Paris (France)

10.40 *Coffee break*

Session VI
moderator Eugenio GAUDIO (WG2 leader)
Oncology Research Institute, Bellinzona (Switzerland)

11.10 O22 **Multi-target paradigm for innovative ligand identification in anticancer drug discovery process**
Christian MULLER University of Strasbourg, (France)

11.30 O23 **Synthetic compound binding to anticancer drug target proteins: thermodynamics and structure of interaction**
Asta ZUBRIENĖ - University of Vilnius (Lithuania)

11.50 O24 **Molecular hybrids targeting 3D Cancer Stem Cells (CSCs)**
Nenad FILIPOVIĆ - University of Belgrade (Serbia)

12.10 O25 **Nur77-induced pro-B cells apoptosis is involving multiple pathways**
Marcel COSTULEANU - University of "Grigore T. Popa", Iași (Romania)

12.30 O26 **Zn(II), Cu(II) and Co(II) complexes with Schiff bases as potential antitumor agents**
Radostina ALEXANDROVA - Bulgarian Academy of Sciences, Sofia (Bulgaria)

12.50 *Lunch*

14.00 **MC meeting (only for the Management Committee)**

14.30 **Poster Session**

Session VII
moderator Maria Laura BOLOGNESI (STSM coordinator)
"Alma Mater Studiorum" - University of Bologna (Italy)

16.00 PL3 **Fluctuations and rare events**
Michele PARRINELLO - ETH-USI, Lugano (Switzerland)

16.40 O27 **Dual COX-2/5-LOX inhibitors inspired by nature**
Marcela DVORAKOVA - Academy of Sciences of the Czech Republic, Prague (Czech Republic)

17.00 *Tea break*

Round table
moderator Fernanda BORGES (CA15135 Vice-Chair) University of Porto (Portugal)

Discussion on research perspectives and future activities of MuTaLig COST Action

- Anna ARTESE (MuTaLig web site manager) - Università "Magna Græcia" di Catanzaro (Italy)
- Dora BOGDAN (MC substitute, organizer of the WG meeting Hungary) - University of Budapest (Hungary)
- Maria Laura BOLOGNESI (STSM coordinator) - "Alma Mater Studiorum" - University of Bologna (Italy)
- Danijel KIKELJ (WG1 leader) - University of Ljubljana (Slovenia)
- Eugenio GAUDIO (WG2 leader) Oncology Research Institute, Bellinzona (Switzerland)
- Sharon BRYANT (WG3 leader) - Inte:Ligand GmbH, Vienna (Austria)
- Hanoch SENDEROWITZ (WG4 leader) - Bar-Ilan University, Ramat-Gan (Israel)
- Maurizio BOTTA (CA15135 Industrial coordinator) - University of Siena (Italy)
- Christa MÜLLER (CA15135 IP coordinator) - University of Bonn (Germany)
- Claire SHOEMAKE (Gender and Inclusiveness coordinator) - University of Malta (Malta)

18.30 **Concluding remarks**

Plenary lectures

Plenary Lecture 1

Patents: applications in the pharmaceutical field

Marco Zardi, Paolo Gerli

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A patent is an exclusive right granted by the State for an invention. To be valid, it must be new, involve an inventive step and be capable of industrial application.

It gives its owner the exclusive right to prevent or stop others from making, using, offering for sale, selling or importing a product or a process, based on the patented invention, without the owner's prior permission.

A patent is granted by the national patent office of a country or a regional patent office for a group of countries. It is valid for a limited period of time, generally for 20 years from the date of filing of the patent application, provided the required maintenance fees are paid on time. A patent is a territorial right, limited to the geographical boundary of the relevant country or region.

In return for the exclusive right provided by a patent, the applicant is required to disclose the invention to the public by providing a detailed, accurate and complete written description of the invention in the patent application.

The scope of the patent protection is defined by the claims, which are of crucial importance during prosecution and infringement procedures. In the field of chemistry, different categories of claims can be found, namely product claims, process claims, claims of intermediate product, product-by-process claims, use claims.

A peculiarity of the patents relating to specific pharmaceutical and plant protection products (e.g. insecticides, and herbicides) is represented by the supplementary protection certificates (SPCs). They provide an extension (maximum 5 years) to a patent's term of expiry and were introduced to compensate for the long time needed to obtain regulatory approval of these products.

Plenary Lecture 2

PI3K inhibition (one Dalton apart)

Doriano Fabbro

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The PI3K signaling pathway is frequently activated in tumors. PQR309, is a selective dual inhibitor of PI3K and mTOR (currently in Phase I) in cancer patients. The preclinical pharmacology and toxicology of PQR309 is presented including its activity in lymphoma pre-clinical models. In addition, we elucidate structural factors defining the PI3K inhibitory activity and tubulin-binding of PQR309 derivatives.

Plenary Lecture 3

Fluctuations and rare events

Michele Parrinello

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In the study of biomolecules as well as in many other fields of science, computer simulations are pervasively used to solve difficult problems. However, very often the systems complexity makes the application of computer simulations challenging. Many systems of interest exhibit long lived metastable states separated by high barriers. In such cases, only very rarely occurring fluctuations allow the system to cross these barriers. This makes the transitions from one metastable state to another rare events. However, although rare, these events are crucial for a correct description of the system. For instance, phenomena such as nucleation, chemical reactions, and protein folding are a few examples of rare events. Unfortunately, the time scale of standard simulation falls short of what needed and the simulation of rare events is one of the main challenges of present day simulations. Here we present a novel approach to this problem, based on the introduction of a variational principle. We show how this variational principle can be used to study complex problems and calculate transition rates of rare events. We underline that besides offering computational efficiency this new approach provides a qualitative new point of view that will have far reaching consequences in the future.

Oral communications

Oral communication 1

The first molecule interacting with a host protein for the inhibition of multiple viruses

Maurizio Botta

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The cellular helicase DEAD-box 3 (DDX3) is known to be an essential host factor for major human viral pathogens such as HIV-1 and Hepatitis B and C viruses as well as for the replication of viral agents responsible for orphan diseases such as Dengue virus (DENV), West-Nile virus (WNV), Human T-cell leukemia Virus (HTLV)-1 and Japanese Encephalitis Virus (JEV). No specific and effective pharmacological treatment is currently available for these latter pathogens, despite being an increasing threat to EU citizens that may eventually lead to sustained epidemics in Europe. Additionally, all compounds that are currently approved for the treatment of other viral infections target viral proteins. Since viruses have evolved the capacity to exploit the cell's molecular machineries as essential components of their replicative cycle, agents designed to interrupt viral replication could, in principle, target with equal effectiveness either a viral or cellular proteins. However, targeting a unique viral function has an important the Achilles' heel: viral resistance to the drugs, an important threat to the efficacy of current therapy. Conversely, the alternative strategy, targeting a cellular factor that is required for viral replication, should help to overcome this problem. Theoretically, a drug targeting a cellular factor could also inhibit all viruses that are dependent on the same host factor. Recently, it has been revealed that the cellular ATPase/RNA helicase X-linked DEAD-box polypeptide 3 (DDX3) is an essential host factor for the replication of several viruses.[1] Accordingly, our research group is working in targeting both the ATPase and RNA binding regions of DDX3.[2-5] Most of the synthesized derivatives were able to inhibit the DDX3 helicase activity at submicromolar concentration. Furthermore, these compounds showed anti-HCV and anti-HIV activity in cells, as well as a good inhibitory activity against JEV, DENV and WNV infections. No cytotoxicity was found for the studied compounds up to 20µM concentration.

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Oral communication 2

Development of Ribonuclease H/DNA polymerase HIV-1 RT dual inhibitors

Simona Distinto,[a] Rita Meleddu,[a] Angela Corona,[b] Giulia Bianco,[a] Filippo Cottiglia,[a] Claudia Melis,[a] Francesca Esposito,[b] Stefano Alcaro,[c] Enzo Tramontano,[b] Elias Maccioni.[a]

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Multi-target drug discovery is an emerging area of increasing interest to the drug discovery community. In fact, drugs rationally designed that modulate several targets have a larger therapeutic window compared to single targets agents. The design of multiple-acting ligands has become a fascinating challenge for the therapy of diseases with multifarious pathological mechanism such as human immunodeficiency virus (HIV) acquired immunodeficiency syndrome (AIDS). The inhibition of multiple targets with a single molecule, in fact, could improve patients' compliance and reduce the occurrence of drug resistance. The DNA polymerase and ribonuclease H (RNase H) activities of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) are needed for the replication of the viral genome, and validated drug targets. However, RNase H remains the only virally encoded enzymatic function for which no inhibitor is available in clinic. Moreover the approved polymerase inhibitors face the occurrence of drug resistance.[1] In this respect the identification of a single molecule able to simultaneously inhibit both functions could represent a significant advance in HIV-1 treatment. Recently, we have reported the identification of HIV-1 RT single site dual inhibitors (SSDIs) by a combined shape-, 2D-fingerprint-, and pharmacophore-based virtual screening approach.[2] Pursuing the strategy of developing new anti-HIV inhibitors we have studied several derivatives[3, 4] and we have hypothesized the possible mechanism by means of both biochemical and computational studies.

References

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[3] Meleddu, R.; Cannas, V.; Distinto, S.; Sarais, G.; DelVecchio, C.; Esposito, F.; Bianco, G.; Corona, A.; Cottiglia, F.; Alcaro, S.; Parolin, C.; Artese, A.; Scalise, D.; Fresta, M.; Arridu, A.; Ortuso, F.; Maccioni, E.; Tramontano, E. Design, synthesis, and biological evaluation of 1,3-diarylpropenones as dual inhibitors of HIV-1 reverse transcriptase. *ChemMedChem*, **2014**, 9, 1869-1879.

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Oral communication 3

Multiple Targets Selection for Design of Inhibitors of Ebola Virus Infection

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The recent outbreak of the Ebola viral disease (EVD) in West Africa reminded us that an effective anti-viral treatment still does not exist, despite the significant progress that has recently been made in understanding the biology and pathology of this lethal disease. The genetic information for the construction of virus are writen on a single-stranded, negative sense RNA, cca 19 k nucleotides in lenght. The EBOV genome encodes following proteins in order from 3' terminus to 5' end: NP, VP35, VP40, GP/sGP, VP30, VP24, and RNA polymerase (L). Many of the Ebola proteins are known for their multifunctionality, for example VP40 controls trafficking , regulates viral transcription and also coordinates virion assembly an budding from infected cells.[1] Despite of its genetic simplicity the Ebola virus has very complex lifestyle which is presented as an ideogram in figure 1:

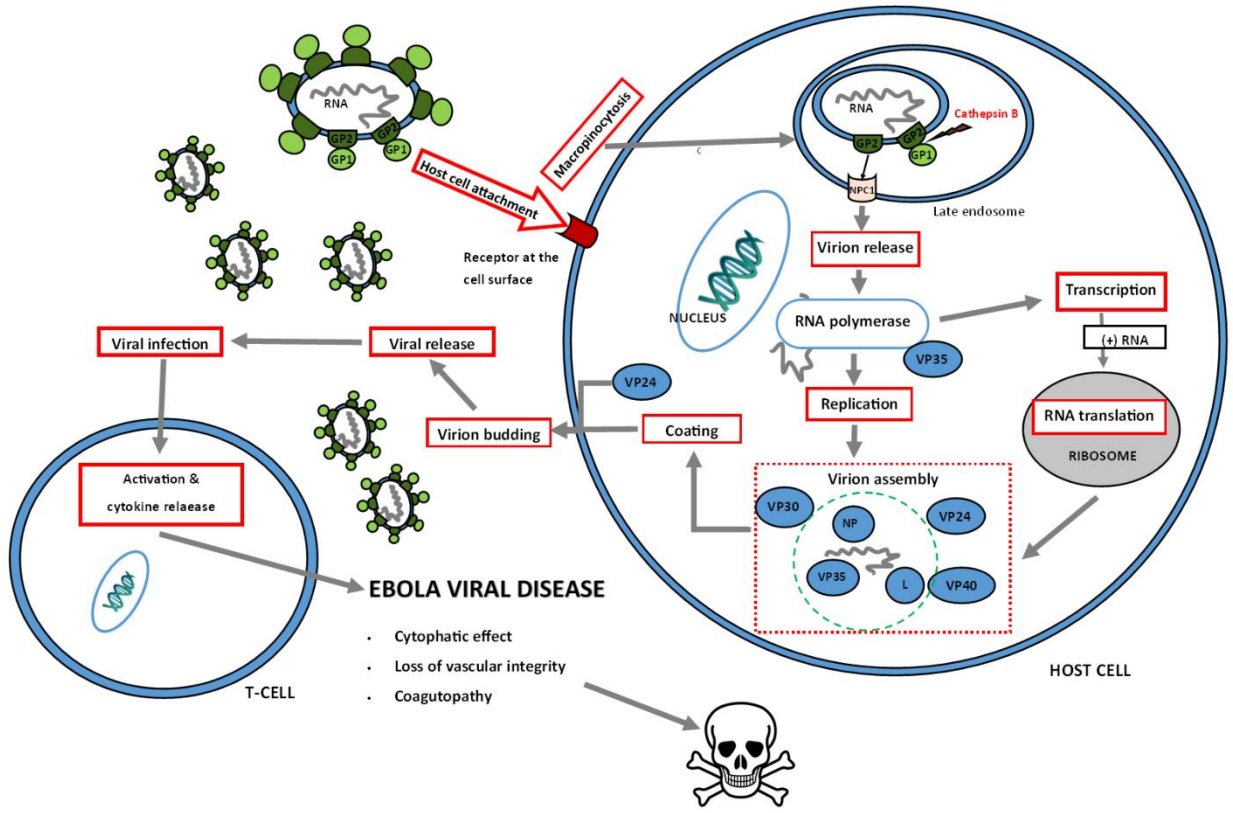

Figure 1: The ideogram of EBOV life cycle along with connections between possible therapeutic targets for the treatment Ebola viral disease.

In this presentation we will review different targets that could be used for structure based design of antiviral agents. The strategy for the design of small molecule inhibitors against the EBOV will include different targets, some of them are EBOV proteins (VP24, VP35, VP40, etc.) and others are host proteins involved in the life cycle of the Ebola virus (Cathepsin B, DC-SIGN, NPC1, ATP1A1, …).[2,3,4] Regarding the mechanism of the Ebola virus several strategies to fight against this virus are possible: (a) Production of monoclonal antibodies that target EBOV glycoprotein; (b) Inhibition of EBOV adhesion to host cell; (c) Inhibition of endosomal escape; (d) Enhancing intracellular innate immunity; (f) Silencing of the expression of EBOV genes by synthetic RNA analogues; (g) Inhibition of viral RNA processing; (h) Disruption of the assembly of viral nucleocapsid and virion budding; (i) Fight against infection by Ebola Virus Like Particle (VLP) delivery.[2]

The recent knowledge about Ebola virus, its life cycle and targets allow us to initiate a proteochemometric modeling PCM. This is a computational method to model the bioactivity of multiple ligands against multiple protein targets.[5] Our future perspective connected to **MuTaLig Cost Action (CA15135)** is to establish a scientific and social network that could accelerate our efforts to find effective medicaments for the Ebola Viral disease.

References

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Oral communication 4

Biofilm targets for the development of novel therapeutics

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It is a natural tendency of microorganisms to attach to abiotic and biotic surfaces, to multiply and to embed themselves in a slimy matrix, resulting in biofilms. They are ubiquitous causing significant problems in the medical area (i.e. they can cause numerous chronic infections, including cystic fibrosis, pneumonia, periodontitis, and varied infections associated with indwelling devices such as catheters, heart valves, orthopaedic devices and contact lenses). The problem is their high-level of tolerance to current therapeutic approaches. In fact, biofilms have been reported as possessing susceptibilities towards antibiotics that are 100 - 1000 times less than equivalent populations of planktonic microorganisms.[1] Moreover, there is no recognized control strategy able to effectively inactivate the colonizing microorganisms and cause their removal from the surface.[1,2] Therefore, new strategies are required to target microbial viability and cause biofilm dispersal. It is known that microorganisms use a number of mechanisms to come into closer contact with the surface, attach firmly to it, promote cell-cell interactions and grow as a complex structure. Those mechanisms are potentially attractive as targets for novel therapeutics. This work will present diverse examples of current anti-biofilm approaches and discuss those key steps on biofilm formation and maintenance representing potential targets for their effective control.

References

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Oral communication 5

Design, Synthesis and Biological Activity of novel dual ATP-competitive inhibitors of DNA Gyrase and Topoisomerase IV

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DNA gyrase and topoisomerase IV are well-known and validated targets of antibacterial drugs. Growing resistance against the fluoroquinolones, that target the catalytic GyrA/ParC subunits, limits their therapeutic potential and stimulates the search for novel inhibitor classes targeting the GyrB/ParE ATP-binding sites. Because of the structural similarities between DNA gyrase and topoisomerase IV, dual targeting is possible in most bacteria, which prolongs the onset of resistance and makes them attractive targets for antibacterial drug discovery.

Recently, we have identified that the 4,5-dibromo-1*H*-pyrrole-2-carboxamide moiety, which is present in *Agelas oroides* marine sponge alkaloid oroidin, is an important moiety for binding to the hydrophobic pocket of the *Escherichia coli* GyrB ATP-binding site.[1] Structure-based optimization of initial low micromolar hits based on the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole scaffold resulted in the low nanomolar *E. coli* and submicromolar *Staphylococcus aureus* DNA gyrase inhibitors possessing also micromolar inhibitory activity of *E. coli* and *S. aureus* topoisomerase IV. Compounds also displayed modest antibacterial activity against Gram positive *S. aureus* and *Enterococcus faecalis*, while they were found to be efflux pump substrates in *E. coli*, which most likely leads to their inactivity against Gram negative bacterial strains.[1]

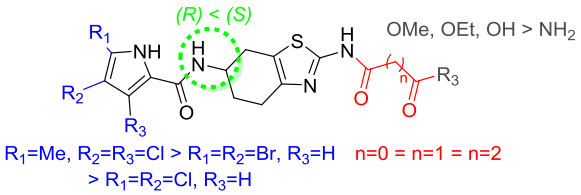

Figure 1: Structure-activity relationship of the novel 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole-based inhibitors of DNA gyrase and topoisomerase IV.

Potent inhibition and observed antibacterial activity highlight these novel structural class of DNA gyrase and topoisomerase IV inhibitors as promising starting points for structure-based design of inhibitors with improved antibacterial activity.

References

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Oral communication 6

Standard and advanced simulations in drug design

Vittorio Limongelli

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Predicting the thermodynamic and kinetic properties of the binding process of a drug to its target is of primary relevance to shed light on its mechanism of action and develop new medications [1,2,3]. Here, I illustrate how this information can be obtained from advanced calculations. In particular, we studied the binding of benzamidine to trypsin using a new approach, called Funnel-Metadynamics [4]. This method enhances the exploration of the ligand bound poses and its solvated states leading to an accurate estimation of the protein-ligand binding free energy. Furthermore, we could recover from metadynamics the kinetic rates of the ligand binding process (k_{off}) using a recently developed protocol [5]. In our simulations, the x-ray conformation was found as the lowest energy pose and the computed ligand binding free energy in good agreement with experiments. Our approach allows also to unveil precious details of the docking process, such as the presence of alternative binding modes and the role of the solvent. Albeit very recent, Funnel-Metadynamics has proven successful in studying complex ligand/protein, ligand/DNA and peptide/membrane binding interactions [6,7,8].

Finally, I present a very recent protocol that combines multiscale and enhanced sampling methods (coarse-grained/metadynamics) to simulate long time-scale events in very large systems [6]. Using such approach the dimerization mechanism in membrane of the transmembrane helices of the epidermal growth factor receptor has been energetically and structurally characterised, shedding light on possible activation pathways of the receptor. Our protocol allows reaching the second time scale, opening new opportunities to study protein clusters in membrane (e.g. GPCRs, ion channels) and protein/protein interactions (e.g. antigen/antibody) in more physiological environment.

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Oral communication 7

3D-Chemical Feature Based Pharmacophores: Essential Tools for Early Drug Discovery Research

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Pharmacophore modeling and virtual screening are techniques widely used by chemists[1] and modellers involved in pharmaceutical research to rapidly visualize and decipher key interaction features between proteins and ligands[2], find biologically active compounds[3], fish for new targets[4], repurpose existing drugs[5], explore protein-protein interfaces[6], and profile drug targets for side-effects.[7] During the last decade we have developed and expanded the capabilities of LigandScout, our molecular design platform, to further support medicinal chemists and modellers in their hit finding, hit expansion, hit to lead, and lead optimization research using advanced pharmacophore methodology. LigandScout is already well known for its ability to automatically derive 3D-interaction feature models starting from a macromolecular-ligand complex.[8,9] In addition, we have developed pattern recognition alignment algorithms for creating models based a set of ligands without active-site information and in active sites where no ligands are present. Furthermore, our current research involves developing advanced methods to analyze molecular dynamics simulation trajectories to create pharmacophore ensembles representing the dynamic event of binding. As an extension of this approach, parallel pharmacophore-based screening has been introduced as an innovative in silico method to predict the potential biological activities of compounds by screening them with multiple pharmacophore models. In the presentation, an overview of our advanced pharmacophore technology developed over the last decade will be given and the results of several success stories presented. Examples range from proof of concept studies employing natural product compounds that were submitted to in silico activity profiling using the Inte:Ligand Pharmacophore Database[10] to in silico fragment-based discovery of novel enzyme inhibitors.

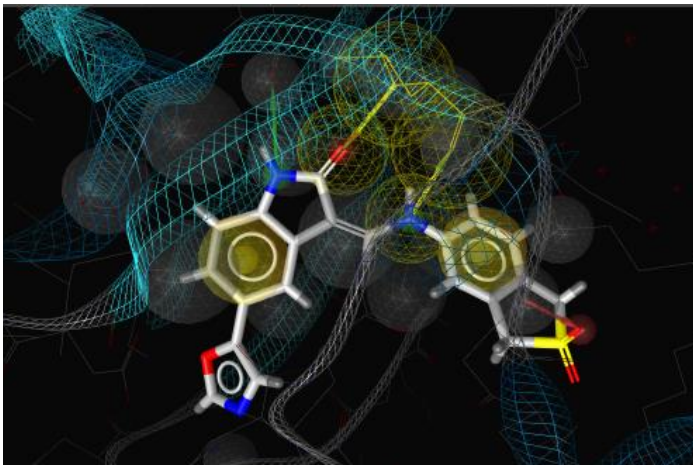

Figure 1: 3D-Chemical feature interaction pharmacophore model automatically derived using LigandScout Advanced 4.0 using cyclin dependent kinase 2 and a kinase inhibitor (x-ray derived complex, PDB Code: 1Ke7). Interactions displayed include yellow spheres (hydrophobic), red arrows (hydrogen bond acceptors), green arrows (hydrogen-bond donors), grey spheres (excluded volumes). Such models can be used for rapid virtual screening of compound libraries to find other molecules with similar interaction features in 3D-space.

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Oral communication 8

Let's start from the very beginning: The virtual screening work flow

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The screening of compounds collections is a widely used starting point in the search for new biologically active compounds. Two types of screening could be performed, either high throughput screening (HTS) or virtual screening (VS). HTS is a resource (e.g., time and money) intensive procedure. These resources could be greatly reduced though VS, namely, the computational screening of thousands to millions of virtual (yet commercially or in-house available) compounds for their binding or activity. The multiple documented successes of VS suggest that today it is a mature paradigm which offers an adequate alternative to HTS efforts. A typical VS workflow operates as follows: A structure of a relevant biological target is obtained from the PDB or generated through homology modeling. A target specific library is obtained by the focusing of large compounds collections available, e.g., from public domains or from different vendors. Focusing is based on the characteristics of the binding site and is typically performed by comparing ligand attributes (e.g., polar surface area, volume, number of H-bond donors and number of H-bond acceptors) with the corresponding binding-site "requirements" and by matching site-derived and ligand-derived pharmacohpores. Compounds surviving the filtration process are docked into the sites using docking algorithms, plausible binding modes are selected and scored and the best scoring ones are purchased and submitted to biological assays. In order to address concerns resulting from known deficiencies of contemporary docking algorithms and scoring functions consensus approaches whereby compounds are docked and scored using multiple tools may be used. Compounds are selected for further processing only if deemed active by the majority of these tools. In the absence of structural information on a relevant target, virtual screening could be performed using pharmacophore models provided that information on active (and preferably also inactive) ligands is available. In such cases library focusing could be based on the attributes of known active ligands.

This seminar will survey the VS workflow suggesting good practices and highlighting potential pitfalls. Special emphasize will be placed on the selection of the screening library since the success of any screening campaign, either high throughput or virtual, critically depends on its quality. Many libraries are currently available from different vendors yet the selection of the optimal screening library for a specific project is challenging. We will present a recently devised workflow for the rational selection of project-specific screening libraries.[1]

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Oral communication 9

Let's develop together an exchange virtual compounds computational platform

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For each lead compound discovered in medicinal chemistry research several other inactive or less active molecules have to be synthetized/isolated. Even if these chemical entities are useful for deriving structure activity relationship with respect to the original target, their development and application stops, in the best cases, in a scientific manuscript or they are forgotten in some storage area. Anyway inactive, or poor active, compounds could live a second youth by testing it with respect to other targets. Such an approach is on the basis of the drugs repurposing and can be carried out by means of virtual screening methods. Several databases, such as ZINC,[1] ChEMBL,[2] DrugBank,[3] NCBI,[4] Phenol-Explorer[5] etc, containing billion of molecules, freely or commercially available, are searchable on the InterNet. These data sources contain structural information and, in some case, activities. Taking into account wide number of participants to the MuTaLig COST ACTION CA15135, the possibility to cross-share compounds and related information could be of interest.

We are proposing the development and the housing of a molecular database, web accessible, where MuTaLig synthetic working group participants can virtually store their compounds accompanied by their activity data, if available. Molecular modeling and biological work groups, for identifying new possible targets, could use stored information. The computational facility will be developed from scratch, so we will define, together, information typology and access policies.

The proposed platform would embody the spirit of the MuTaLig COST ACTION CA15135 and could be useful for repurposing already available compounds highlighting tentative multi-acting molecules.

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Oral communication 10

Discovering and designing new drugs considering toxicity and safety from the early stages incorporating multiple targets and anti-targets

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In silico virtual screening is one of the first steps in compound development and can include a variety of target structures, as well as prediction and profiling of off- and anti-target effects. The use of a combination of modified techniques, chemical libraries, and protein structures[1] resulted in novel, low-toxic, nanomolar *s*-triazine inhibitors of HIV-1 infection and of HIV-1 reverse transcriptase (RT).[2] In a separate project, re-ranking resulted in inhibitors of HIV-1 infection and of HIV-1 integrase (INT).[3] Another case involves the design of compounds for neglected diseases using multiple targets. The anti-targets were adapted into a matrix composed of a modified score of interactions with proteins involved in the metabolism of substances, such as the pregnane-X-receptor, sulfotransferase, cytochrome P450 2a6, 2c9, and 3a4. The chemical libraries so composed, can be useful to design compounds with an early flagging of possible interactions, in order to incorporate safety as early as possible into the drug design process.

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Oral communication 11

Development of dual- and multi-target drugs for the treatment of neurodegenerative diseases

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Neurodegenerative diseases, such as Parkinson's (PD) and Alzheimer's disease (AD), are complex, multifactorial disorders of the brain, characterized by inflammatory processes, protein aggregation, and loss of neuronal functions. Currently, only symptomatic treatments are available. Research efforts are aimed at developing disease-modifying drugs, which can slow down or even stop disease progression. Single-target drugs appear to be insufficient for the treatment of such complex diseases as illustrated by a considerable number of failures of this type of drugs in clinical trials. Therefore, the concept of dual- and even multi-target drugs interacting with more than one target, has been proposed for complex diseases, including cancer, psychiatric disorders, and also neurodegenerative diseases.[1]

The consumption of caffeine, a weakly potent, non-selective **adenosine receptor** (AR) **antagonist**, protects from AD and PD as shown in large epidemiological studies and in a number of animal models.[2] **Monoamine oxidase** (MAO) type B is the major MAO in the human brain, which was found to be upregulated in PD and AD. MAO-B inhibitors are co-applied with levodopa to increase the bioavailability of dopamine in PD patients. At the same time MAO-B inhibitors decrease the formation H_2O_2, a side product of the MAO-B reaction. Therefore, MAO-B inhibitors may exhibit neuroprotective properties.[3]

We have developed different series of dual- and triple-target drugs with the following expected properties: (i) MAO-B inhibitors (improvement of motor symptoms in PD patients; potentially neuroprotective due to reduced amount of reactive oxygen species in PD and AD; (ii) A_1AR antagonists (improved cognition in AD and PD); (iii) A_{2A}AR antagonists (improved motor symptoms in PD; neuroprotection and improved cognition in AD and PD). Our approach has been to design small molecules that can be used for preclinical proof-of-principle studies and may be suitable for peroral therapy in humans.[4]

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Oral communication 12

Development of dual target adenosine A_1/A_{2A} receptors antagonists among annelated xanthine derivatives

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Drugs that act at multiple targets and provide symptomatic benefits as well neuroprotective effects have been proposed to be advantageous for the treatment of neurodegenerative diseases like Parkinson's and Alzheimer diseases. Dual A_1 and A_{2A} AR antagonists were found to be highly active in different models of PD and AD. It was reported that dual A1/A2A ARs antagonists improved neuronal degeneration and motor impairment via A_{2A} AR blockade and showed positive effects on the cognitive impairment associated with the disease by blocking A_1ARs. So dual target adenosine A_1/A_{2A}ARs antagonists represent attractive drug candidates for the treatment of neurodegenerative disorders[1-3].

In our efforts[4-6] to develop potential drugs for the treatment of neurodegenerative disorders annelated derivatives of dibutyl and dipropyl xanthines with amide moiety were designed and synthesized. Aminoalkyl amide derivatives of 4-hydroxy, 3-hydroxy phenyl- and 4-hydroxy phenethyl 1,3-dibutyl or 1,3-dipropyl substituted pyrimido[1,2-*f*]purine-2,4-diones were obtained. Compounds were evaluated on their activity against A_1, A_{2A}. A_{2B} and A_3 ARs. A_1/A_{2A} AR ligands with Ki values at nano and submicromolar range were identified. Structure-activity relationship analyses have shown that activity depends on the kind of amine moiety and substituents at N1 and N3 position. Drug-like properties of the examined compounds were estimated.

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Oral communication 13

Anitimicrobial activity of 1,8-naphthalimide metal complexes

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In the last years the coordination chemistry is highly developed due to the possibility of creating complexes of metal ions with organic molecules. The design of specific ligands that can coordinate with metal ions is a major goal of many researchers. On the other hand, the biological activity of the metal complexes is highly dependent on the nature of the metal ions and the ligands because different ligands exhibit different biological properties. Although some metal complexes with organic ligands exhibit good antibacterial and antifungal activity, research continues in search of new more effective antibacterial and antifungal compounds. Some 1,8-naphthalimides derivatives exhibit good therapeutic activity against viruses, fungi and different types of bacteria. Recently, our group studied the mode of coordination and antimicrobial activity of Cu(II) and Zn(II) 1,8-naphthalimide complexes (Figure 1).

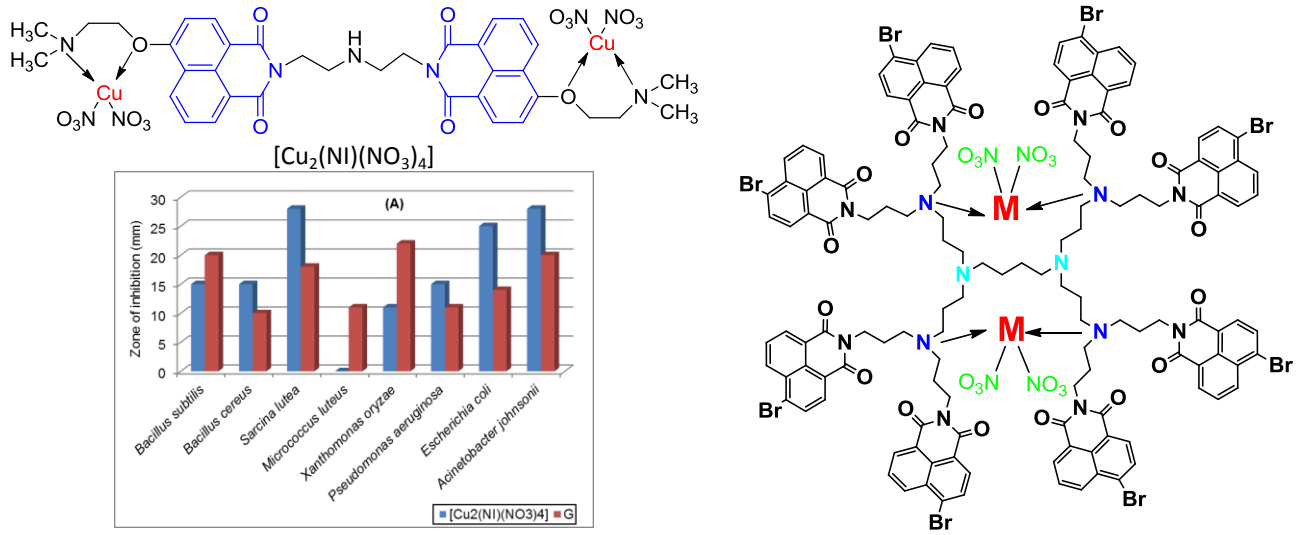

Figure 1: Chemical structures and antibacterial activity of Cu(II) and Zn(II) 1,8-naphthalimide complexes

In this presentation the functional properties of some new 1,8-naphthalimide derivatives and their Cu(II) and Zn(II) complexes are presented. *In vitro* antimicrobial screening of the newly synthesized complexes has been carried out against some pathogenic Gram-positive and Gram-negative bacteria and yeasts. The results showed than the new metal complexes exhibit good antibacterial and antifungal activity against the tested pathogens. Also it has been shown that the molecular weight is an important parameter that influences antimicrobial activity of the 1,8-nafthalimides.

Oral communication 14

Inhibitors of aldose reductase with multi-target properties

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Aldose reductase (ALR2) is a promissive therapeutic target in treating long-term diabetic complications and other chronic diseases. We present a survey of our research on aldose reductase inhibitors: design as well as in vitro and in vivo testing of their efficacy.

Indole-1-acetic acid moiety is known as a promising starting fragment for design of efficient aldose reductase inhibitors. Our first prospective compound arised from the screening of databases of purchasable compounds. Fifteen compounds, having indole-1-acetic acid moiety as a common fragment in their structure, were selected for an experimental assessment. Among the compounds studied, 5-carboxy-3-mercapto-1,2,4-triazino-[5,6-b]indole (CMTI) was identified as the most promising inhibitor of aldose reductase (ALR2), with IC_{50} = 97 nM and selectivity factor relative to aldehyde reductase around 400. The position of CMTI in ALR2 has been identified and deposited in protein database as the crystal structure with pdb code 4qx4 (Fig. 1).

Based on our previous experience with beneficial role of pyridoindoles in pathological processes connected with oxidation stress, we designed and tested several carboxymethylated compounds with hexahydro- and tetrahydro-pyridoindole structure. During this study we found further promissive compounds with the inhibition activity and selectivity even more advantegous for further preclinical testing.

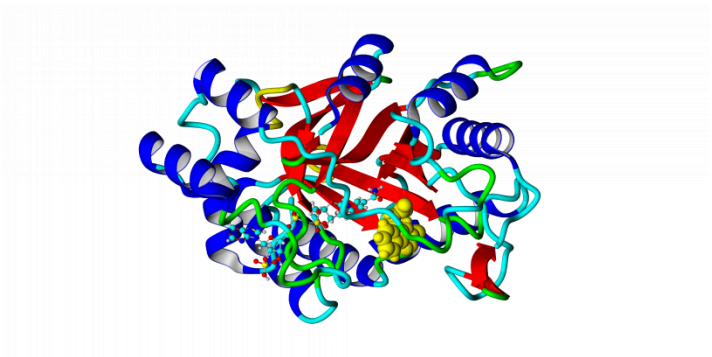

Figure 1: Crystal structure of ALR2 complexed with CMTI (pdb code 4qx4)

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Oral communication 15

Mitochondrial targets for multi-target ligand design

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Mitochondria are vital to energy production in mammalian cells and control signals for apoptosis. In terms of targets for drug design, a key cytosolic-facing enzyme is monoamine oxidase (MAO). MAO A and B have different distributions and substrate specificity but both are vital for the metabolism of any amine whether neurotransmitter or xenobiotic or therapeutic drug. Screening for MAO inhibition is not straightforward, so some of the interesting physiological and chemical features of these enzymes will be discussed, including examples from recent multi-target compounds designed to combat Alzheimer's Disease[1, 2.] However, the function of mitochondria as energy producers is key to the health of the cell and its ability to withstand oxidative stress. New technology to measure oxygen consumption and bioenergetics parameters in living cells enables study of mitochondria within cultured cells. Fully differentiated SH-SY5Y neurons treated to decrease cell viability were protected by MAO inhibitors. The stressor increased the proportion of rounded and interconnected mitochondria but MAO inhibitors gave some protection. Interactions with cytosolic proteins controlling these dynamics will next be explored using proteomics[3]. In summary, MAO inhibitors are able to protect against HCA-induced neurotoxicity. These effects indicate that the mechanism of MAOI in the treatment of neurodegeneration is not just to spare the neurotransmitters.

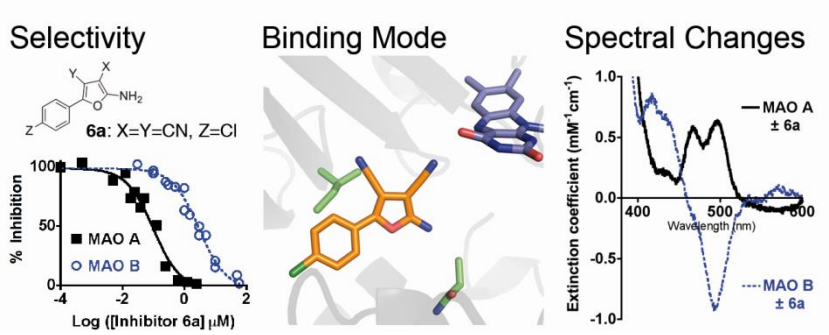

Figure 1: Kinetic, spectroscopic and computational studies help optimize inhibition of MAO.

References

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Hydroxycinnamic acid as a valid scaffold for the development of multitarget ligands for CNS disorders

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Hydroxycinnamic acids (HCAs), a class of naturally-occurring antioxidants endowed with multiple *in vitro* pharmacological activities, are a privileged scaffold for the development of multitarget ligands for CNS disorders.[1] Based on this background, a small library of lipophilic HCA derivatives was designed to meet the main pharmacokinetic requirements to cross the BBB, interact with multiple targets within the CNS and mitigate the formation of reactive metabolites. The derivatization of the HCA scaffold with a 5-nitro substituent yielded potent and BBB-permeable catechol *O*-methyltransferase (COMT) inhibitors within a nanomolar range comparable to that of the standard COMT inhibitors tolcapone and entacapone. These derivatives also showed *in vitro* tau aggregation inhibition and copper metal chelation. The presence of α-nitrile groups and carboxamide functions in the side chain greatly improved tau aggregation inhibitory activity. The 5-nitro HCA derivatives did not show significant hepatotoxicity in rat primary hepatocytes at 50 µM, in contrast with the effects observed for entacapone and tolcapone. SH-SY5Y cells were the most susceptible to nitrocatechol-induced toxicity, particularly in their undifferentiated phenotype, and this effect was not mediated by overproduction of reactive oxygen and nitrogen species. Nitrocatechols showed altered redox behaviour, mainly regarding oxidation reversibility and the stability of reductive hydroxylamine metabolites. Lipophilicity was a key determinant of the cytotoxicity observed in undifferentiated SH-SY5Y cells, but not in differentiated SH-SY5Y cells, Caco-2 cells and rat primary hepatocytes. Cytochrome P450 metabolism and P-gp-mediated phase III efflux did not influence the cellular effects of the studied 5-nitro HCA derivatives. The mitochondrial toxicity profile of the most promising compounds was either similar to or more favourable than that obtained for the non-hepatotoxic COMT inhibitor entacapone. Due to its BBB permeability, transition metal chelation capacity, potent inhibition of COMT and tau aggregation inhibitory activities, increased safety over tolcapone and straightforward potential for chemical modification, CNCAPE (Figure **1**) was proposed as a multitarget lead for further optimization, with potential therapeutic applications for CNS disorders.

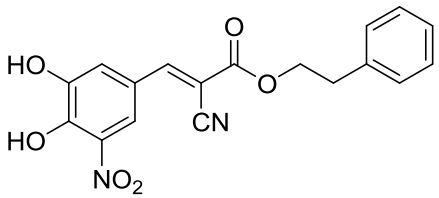

Figure 1. Chemical structure of CNCAPE.

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Oral communication 17

3D-QSAR and design of dopamine D3 receptor ligands

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Development of potent and selective dopamine D3 receptor (D3R) antagonists/partial agonists has been challenging because of high sequence homology between the D3R and the dopamine D2 receptor (D2R). In an effort to design selective D3R ligands the data set of 86 compounds acting as D3R and D2R ligands[1] has been examined by 3D-QSAR study. The crucial molecular determinants (Figure 1.) specific for high D3R and low D2R affinity have been identified by 3D-QSAR pharmacophore models (3D-QSAR (D3R): Q^2(0.73), RMSEE(0.409), R^2(0.842), RMSEP(0.475), R^2_{test}(0.707); 3D-QSAR (D2R): Q^2(0.75), RMSEE(0.408), R^2(0.86), RMSEP(0.381), R^2_{test}(0.726)).

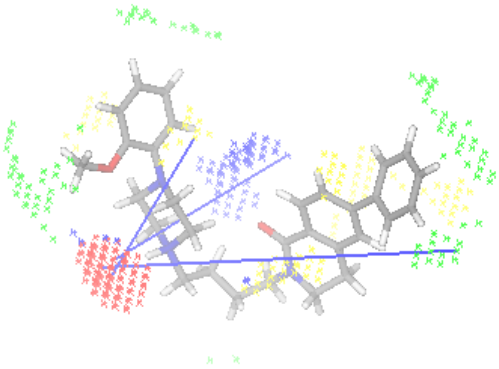

Figure 1: The crucial molecular determinants specific for high D3R affinity

Created 3D-QSAR models were applied for design and selection of the most promising D3R antagonists/partial agonists. Designed compounds with 3D-QSAR predicted p*K*i (D3R)>8.8, selectivity (p*K*i(D3R)-p*K*i(D2R)>1.5, and ClogP <5.0. were selected for further profiling. Ligand-based virtual screening of ZINC database, data set and designed compounds is performed against selected lead molecule. Eight designed D3R ligands, ranked within top 30 compounds, were selected as the most promising candidates for further studies.

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Multi-Objective Optimization in Drug Design

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Modeling complex phenomena in chemoinformatics and drug design can often be formulated as multi-variables/single objective or multi-variables/multi-objectives optimization problems (SOOP and MOOP, respectively). In particular, the design of new compounds with pharmaceutical relevance is inherently a multi-objectives optimization problem since drugs require the simultaneous optimization of many, sometimes conflicting parameters.

The formulation of optimization problems requires the definition of a target (or objective) function (*f*) and a set of variables (x_1, x_2, x_3,…x_n), which are related to the scientific problem of interest, and which together define a complex, multi-dimensional surface with an a priori unknown distribution of optima. The task then is to locate the global optimum or preferably, since phenomena in these fields are rarely governed by a single solution, a set of optima. Optimization problems could be broadly divided into two categories namely, single objective optimization problems and multi-objectives optimization problem depending on the number of target functions which should be simultaneously optimized. Due to the complex nature of many of the chemoinformatics related target functions (e.g., non-linearity, non-continuity, non-derivability), a global stochastic optimization algorithms should be used as the optimization engine. Several such algorithms were reported in the literature including simulated annealing and genetic algorithm. However, while the role of optimization techniques in drug design and chemoinformatics has been growing over the last few years, their full potential in these fields is far from being thoroughly explored.

In this seminar the principles of multi objective optimization will be presented together with two approaches for solving multi objective optimization problems, the Pareto front method and the transforming of MOOP into SOOP method. In addition several global optimization algorithms will be presented including Monte Carlo / Simulated Annealing and Genetic Programming and their usage in solving MOOPs will be discussed. Finally, multi-objectives optimization-based solutions for two chemoinformatic-related problems, the selection of a representative subset from within a parent database[1] and the removal of outliers[2] will be presented. We propose that treating drug development as a MOOP is highly relevant to the MuTaLig COST action.

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Oral communication 19

The MEK-inhibitor pimasertib is synergistic with PI3K-delta and BTK inhibitors in lymphoma models

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Pimasertib (AS-703026) is a potent and highly selective ATP noncompetitive MEK1/2 inhibitor that has shown anti-tumor activity in different pre-clinical models. We have previously reported pimasertib activity as single agent in lymphoma models and preliminary combinations results (Gaudio et al, AACR 2014). Here, we report detailed data on combinations of pimasertib with the PI3K-delta inhibitor idelalisib and with the BTK-inhibitor ibrutinib.

Methods. Cell lines derived from activated B-cell like (ABC) diffuse large B-cell lymphoma (DLBCL) (OCI-Ly10, TMD8), germinal center B-cell like (GCB) DLBCL (DOHH2, RCK8) and from mantle cell lymphoma (REC1, JEKO1) were exposed to increasing doses of pimasertib alone and in combination with idelalisib and ibrutinib. Synergy was assessed by Chou-Talalay combination index (CI). ERK phosphorylation level and PARP cleavage were detected by western blotting in cell treated with single agents or combination of pimasertib with idelalisib or ibrutinib. NOD-Scid (NOD.CB17-Prkdcscid/NCrHsd) mice were subcutaneously inoculated with OCI-Ly-10 (10 x106) DLBCL cell line. Mice developed palpable tumors (100 mm3) and were randomized to receive pimasertib, orally once per day (30 mg/kg), ibrutinib (5 mg/Kg), the combination of the two, or control vehicle alone. Tumor size was measured two times per week using a digital caliper [tumor volume (mm3) = (I x W x W)/2].

Results. Strong synergism was observed with pimasertib combined with the PI3K inhibitor idelalisib in the ABC-DLBCL OCI-Ly10 (median CI= 0.026) and TMD8 (CI= 0.25), whereas synergistic/additive effects were detected in GCB-DLBCL (DOHH2, RCK8) and MCL (REC1, JEKO1). Synergism was observed with the BTK-inhibitor ibrutinib in all the cell lines: OCI-Ly10 (CI= 0.32), TMD8 (CI=0.63), DOHH2 (CI= 0.66), RCK8 (CI= 0.87), REC1 and JEKO1 (CI= 0.2). Thirty minutes of exposure time with pimasertib were sufficient to knock-down phospho-ERK1/2 proteins in the mentioned ABC-DLBCL and MCL cell lines stimulated with anti-IgM (20µg/mL) for 20 minutes. Stronger down-regulation of phospho-ERK1/2 was seen in ABC-DLBCL and MCL cell lines treated with combination of pimasertib with idelalisib or pimasertib with ibrutinib rather than single agent treatment conditions. Notably, apoptosis and PARP cleavage was observed in cell lines treated with pimasertib in combination with ibrutinib or idelalisib. OCI-Ly10 xenograft tumors that received a combination of pimasertib (30mg/Kg) and ibrutinib (5mg/Kg) for 14 days, showed a five-fold reduction of both tumor volume and weight as compared to the control and single compound groups.

Conclusions. Pimasertib-containing combinations with PI3K-delta and BTK inhibitors show very promising activity in preclinical models of mature lymphomas.

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Isoellipticine: targeting cell proliferation by a structured approach

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Ellipticine (Figure 1) is a nautral product which possesses multimodal anticancer activity. Some of its modes of action include DNA intercalation, topoisomerase II inhibition, p53 modulation and formation of cytotoxic adducts. While ellipticine itself is not suitable for therapeutic use, two derivatives, 2-methyl-9-hydroxyellipticinium acetate and 2-[2-(diethylamino)ethyl]-9-hydroxyellipticiunium chloride have progressed to clinical trials. The production of analogues with increased selectivity and potency remains a significant challenge but recent research highlights its versatility.[1-3]

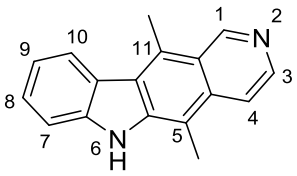

Figure 1. Ellipticine

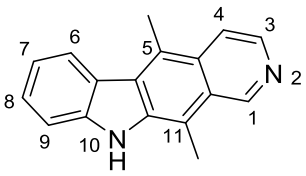

Figure 2. Isoellipticine

The introduction of substituents at the N6 and C9 position have been shown to increase the cytotoxic activity of the molecule. Therefore we have undertaken substitution of an isomer of ellipticine, isoellipticine (Figure 2.) with particular emphasis on analogues substituted at the C7 and N10 positions. Initially, the synthesis of isoellipticine was carried out using a route devised by Gribble with novel routes explored as a means to increase the yield.[4] While N2 substituted analogues have been shown to have varying levels bioactivity, further modification at the N10 and C7 positions has led to excellent activity.[5] We have identified that 7-substitutued isoellipticines can cause G2/M arrest in MV4-11 cells, Molt-3, K563 and HL60 among others.[6] We will present the details of the synthesis of isoellipticine derivatives as well as their anticancer activity.

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Oral communication 21

Identification of new multi-targeted molecules, designed as Neuropilin antagonists, with potent anti-angiogenic and anti-tumour activity *in vivo*.

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Neuropilin-1 (NRP-1) is a VEGF-A165 extra-cellular co-receptor involved in angiogenesis and in the progression of tumour growth. Its over-expression is related to a clinical poor prognosis. By means of a multi-step drug selection procedure performed on 500000 compounds, and which includes structure based virtual ligand screening (SB-VLS) then cellular and molecular assays, we identified 3 promising NRP antagonists. *In vitro*, these molecules exert not only potent anti-angiogenic activity, but also anti-proliferative effect on a large panel of human solid and haematological cancer cell lines. Moreover, they reduce the tumour growth of MDA-MB-231 human xenografted NOG mice (more than 80% at a dose of 50 mg/kg), enhance significantly mice survival, and does not exhibit any acute toxicity. Altogether these results highlight their huge potential for further development of new anti-tumour drugs. Nonetheless, the biological profile of these compounds revealed also: (i) stronger cellular effects than molecular effects and (ii) cytotoxic effects on cells which does not express NRP-1. This suggests that these molecules are multi-targeted ligands, and we believe that the COST MuTaLig consortium might help us to characterize their way-of-action.

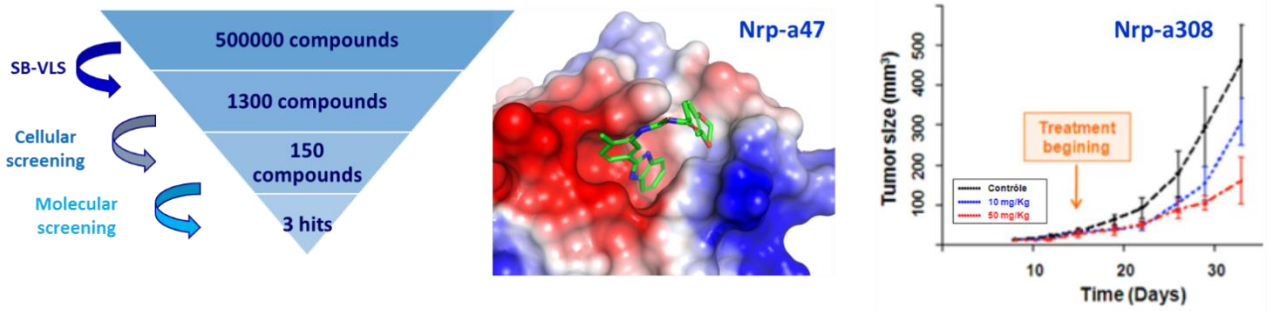

Figure 1. Drug selection procedure (left panel); Nrp-a47 docking into Nrp-1 (central panel); *In vivo* study of Nrp-a308: Evolution of tumor size on NOG mice xenografted with human MDA-MB-231 breast cancer cells (right panel).

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Multi-target paradigm for innovative ligand identification in anticancer drug discovery process

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Antitumor efficacy in preclinical cancer models does not translate faithfully to patient outcomes as drug discovery is mostly performed in cell-based models that poorly represent real malignancies. 3D cultures of different human cell lines (testis & pancreatic CSC, blood & liver carcinomas) are a new anti-cancer therapeutic approach to study in detail differential activities. Testing in checkerboard (fig 1) different sets of drugs will allow determining lowest efficient concentrations. Several sets of compounds in combination or not with traditional drugs will be studied.

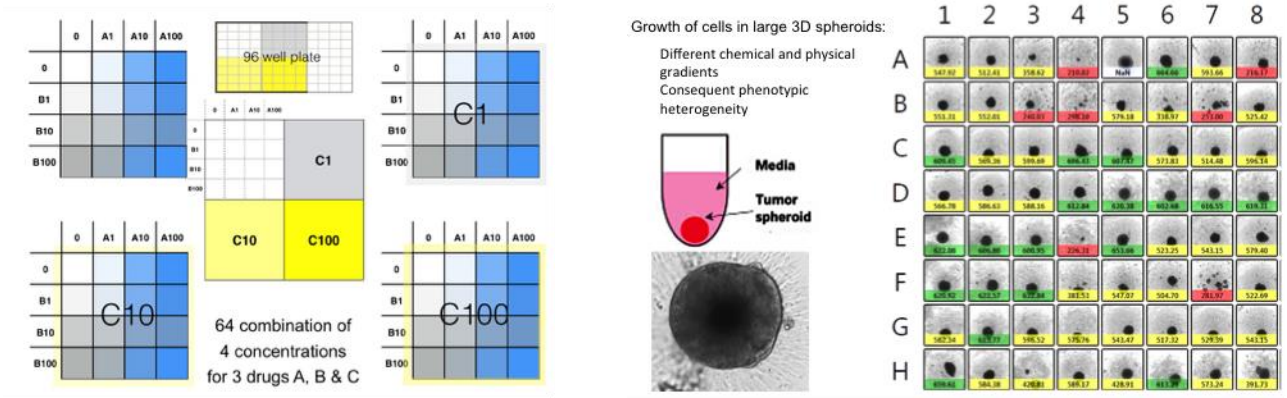

Figure 1: Checkerboard format for 3 drugs combinations - spheroid of cancer cells - typical results obtained

Original hybrid drug molecules [1,2] will be designed in Serbia to counterbalance side effects associated with other hybrid part or to amplify its effect or to interact with multiple targets thus lowering the risk of drug-drug interactions and minimizing drug resistance. In conclusion the risk of failure of this project is minimized due to the choice of in vitro three-dimensional culture systems of tumor cells.

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Oral communication 23

Synthetic compound binding to anticancer drug target proteins: thermodynamics and structure of interaction

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Protein-ligand interactions are one of the main objects of study in protein biophysical chemistry. Molecular forces and underlying reasons that distinguish marginal from high affinity or selectivity as well as the impact of water are not well understood. Thermodynamic characterization of protein–ligand interaction is an important step in drug discovery and optimization of a hit to lead development[1]. Deep understanding of the energetic forces driving the binding provides valuable information for further drug development. The combination of isothermal titration calorimetry (ITC) and fluorescence-based thermal shift assay (FTSA) provides a robust estimate of the binding constant.

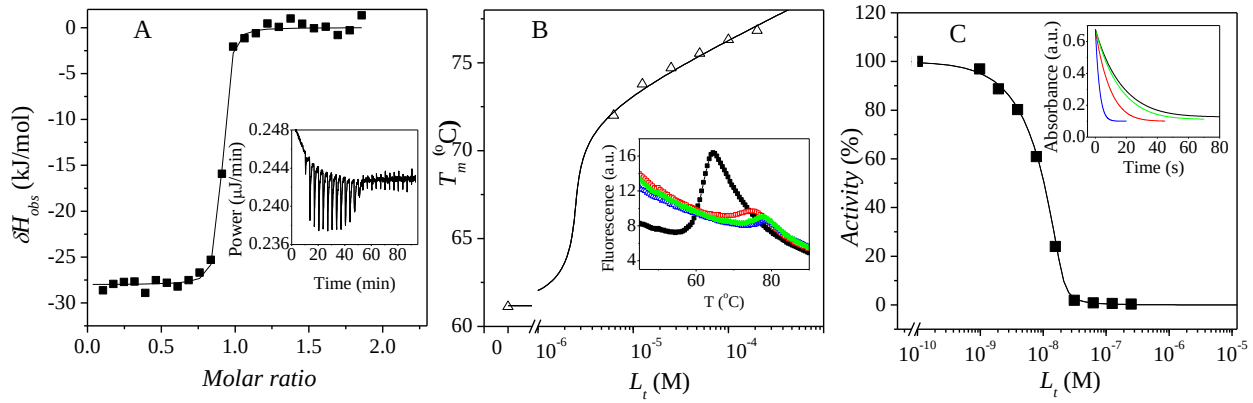
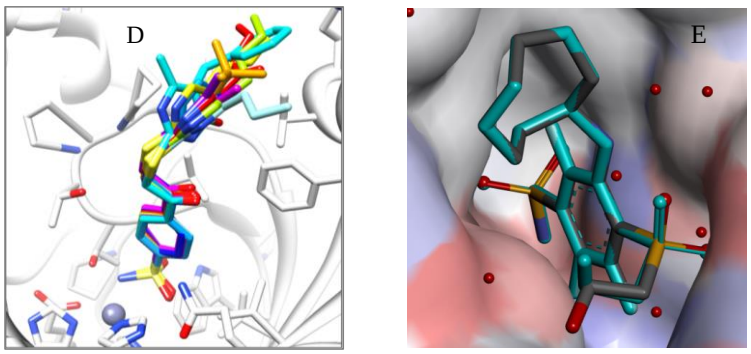


Figure 1. Isothermal titration calorimetry (A), fluorescent thermal shift assay (B) and enzymatic inhibition assay (C) for ligand binding to carbonic anhydrase IX (adapted from Dudutiene et al[2]). In silico docking (D) and X-ray crystal structures of compounds in complexes with carbonic anhydrases (E).



FTSA measures the binding constant of a ligand by determining the increase in the melting temperature of the protein upon ligand binding. Protein melting temperature shift correlates with binding affinity. ITC measures binding reactions by the detection of the heat change during the ligand binding and provides complete thermodynamic characterization of the binding of drug to its target, including enthalpy, entropy, and Gibbs free energy. Binding affinity data will be compared with enzymatic inhibition methods using anticancer targets, carbonic anhydrases, heat shock protein 90 (Hsp90), and several epigenetic therapeutic targets. Combination of the thermodynamic parameters together with the crystal structures of compounds bound to target protein provide the direction of optimization of the compound binding affinity and selectivity towards desired enzyme isoform. The structure-thermodynamics correlations together with molecular modelling are useful for the design of drug-like lead compounds with desired binding properties.

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Oral communication 24

Molecular hybrids targeting 3D Cancer Stem Cells (CSCs)

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The subject of interest of Todorović's group is synthesis of various anticancer molecules containing different pharmachophores such as chalcogensemicarbazones (ribouncleotide reductase inhibitors),[1] thioazoles (kinases, histone deacetylase, matrix and BCL-2 proteinases inhibitors)[2] and selenoazoles (apoptosis inducers)[3]. Also, in collaboration with Silvestri's group, metal complexes with indole containing tubulin polymerization inhibitors were prepared and it was found that they also target DNA.[4] Finally, we have started to work in the field of synthesis and investigation of anticancer activities of thiocarbohydrazones. This class of compounds induced apoptosis in CSC lines. These results were obtained in collaboration with Dr Muller. EIIP calculation showed structural similarities between investigated thiocarbohydrazones and well known inhibitors of cyclin dependent kinases, such as 4TTH and fisetine, while docking studies showed that some of these molecules have even higher binding energies.

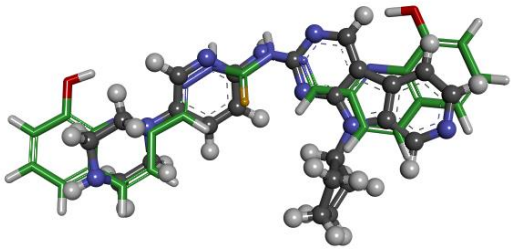

Figure 1. Overlapped structures of cyclin dependent kinase inhibitor 4TTH and thiocarbohydrazone TCH 41.

In the current project we work on design and synthesis of dual molecular hybrids (MH) which contain mentioned pharmachophores. Novel MH will be tested on THP-1 cell line, while the most active MHs will be tested on 3D AsPC-1 CSCs.

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Oral communication 25

Nur77-Induced pro-B cells apoptosis is involving multiple pathways

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Beside its regulatory function on transcription, Nur77 is directly inducing apoptosis by mitochondrial targeting, where it can convert Bcl-2, an anti-apoptotic protein, into a proapoptotic molecule. The aim of our studies was represented by the effects of Nur77 activation on Ba/F3 apoptosis. Ba/F3, a murine interleukin-3 dependent pro-B cell line, became increasingly popular as a model system for assessing both the potency and downstream signaling of kinase oncogenes, and the ability of small-molecule kinase inhibitors to block kinase activity[1]. For some experiments (in triplicate), Ba/F3 cells (gift of Dr. Zonda from IFOM-IEO Milan) were treated with 10 μM Cytosporone B, a natural activator of Nur77, for 24 hours. To compare, we used as control the effects of Valinomycin 1 μM, a well known inducer of dissipation of mitochondrial membrane potential (equal apoptosis), also in triplicate. Separate batches of Ba/F3 cells, in triplicate, were pre-treated with 1 μM Cyclosporine A (inhibitor of mitochondrial permeability transition pore, as well as of calcineurin), 10 μM LY-294,002 (PI3K and PI4K inhibitor), 1 μM PD-98,059 (specific inhibitor of the activation of mitogen-activated protein kinase kinase, MAPKK), and 1 μM Deltamethrin (calcineurin inhibitor). After that, all batches of Ba/F3 cells were incubated in the presence of 1 μM JC-1 (Sigma-Aldrich) at 37°C for 30 minutes. JC-1 [5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide or $CBIC_2(3)$] is a very sensitive marker for mitochondrial membrane potential[2]. The mitochondrial membrane potential dissipation was measured using FACS and confocal microscopy. Cytosporone B (10 μM), Cyclosporine A (1 μM), LY-294,002 (10 μM), PD-98,059 (1 μM), as well as Deltamethrin (1 μM) alone did not significantly induced induced apoptosis of Ba/F3 cells. LY-294,002 (1 μM) induced apoptosis of Ba/F3 cells. On the other hand, Cyclosporine A (1 μM) facilitated the Cytosporone B (10 μM)-induced apoptosis, with an increase of about 60%. The result is astonishing, knowing that Cyclosporine A is a clear inhibitor of mitochondrial permeability transition pore. Its inhibitory effects on calcineurin might be involved. Therefore, another calcineurin inhibitor, Deltamethrin, had no facilitatory effects on Cytosporone B. Very interesting, the apoptotic effects of Cytosporone B + Cyclosporine A were reversed by 10 μM LY-294,002, a concentration with no effects by itself. Finally, PD-98,059 (1 μM) was also without effects on Cytosporone B or Cytosporone B + Cyclosporine A. In conclusion, the apoptosis mediated by Nur77 activation in pro-B cell line Ba/F3 is a complex process and needs several targeting pathways to be studied in order to decipher its fine mechanisms and be addressed by pharmacologic approach.

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Oral communication 26

Zn(II), Cu(II) and Co(II) complexes with Schiff bases as potential antitumor agents

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Various Schiff bases and their metal complexes have been reported to posses interesting biological activities.[1] One of the remarkable advantages of this type compounds is their potential ability to react with different cellular/molecular targets. The aim of our study was to evaluate the influence on tumor cell viability and proliferation of 12 newly synthesized metal complexes with Schiff bases: four Zn(II) complexes with Schiff bases obtained by a condensation reaction between 2,6 diformyl-p-cresol with Alanine, Valine, Leucine or Phenylalanine and eight complexes of Cu(II) (four) and Co(II) (four) with Schiff bases derived by a condensation reaction of *o*-Vanillin with Tyrosine, Threonine, Tryptophan or Serine. As model systems were used three permanent human cell lines – HeLa (carcinoma of the uterine cervix), MCF-7 (hormone dependent breast cancer) and MDA-MB-231 (triple negative breast cancer). The cell lines MCF-7 and MDA-MB-231 differ by the expression levels of some receptors (for estrogen, progesterone, epidermal growth factor), p53 gene (wild type and a mutant variant) and invasiveness. The investigations were performed by short-term experiments (24-72h, with monolayer cultures) and long-term experiments (20-30 days, with 3D cell colonies) including methods with different molecular/cellular targets and biochemical pathways - MTT test, Neutral red uptake cytotoxicity assay, Crystal violet staining, Double staining with Propidium iodide and Acridine orange, TUNEL test, Annexin V – FITC test, Colony forming technique. Our results indicate that applied at a concentration range of 10 – 200 µg/ml the examined metal compounds decrease cancer cell viability and/or growth and induce cytopathological changes. The observed cytotoxic/cytostatic effect has been found to be time- and concentration-dependent. Co(II) complexes exhibit lower cytotoxic/antiproliferative activity as compared to Zn(II) and Cu(II) complexes. Additional investigations are required to clarify better the anticancer potential of these compounds as well as their cellular targets and mechanism(s) of action.

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Oral communication 27

Dual COX-2/5-LOX inhibitors inspired by nature

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We would like to synthesize dual COX-2/5-LOX inhibitors based on our previous results obtained from the isolation and identification of anti-inflammatory compounds from plants. Several natural compounds possessing an interesting *in vitro* activity either on COX or LOX enzymes were identified (Figure 1).[1-3] Among them, especially quinonic compounds and dietary stilbenes exhibited promising inhibitory activity on COX and 5-LOX enzymes.[1]

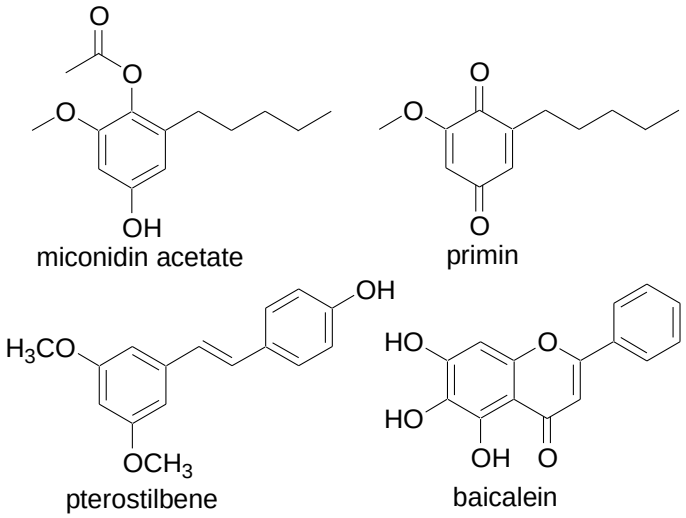

Figure 1: Example of natural products with COX or 5-LOX activities

We would like to modify the structures of natural COX/5-LOX inhibitors using appropriate bioisostere units to enhance their inhibitory activity and decrease their toxicity, which is the main concern especially in case of quinonic compounds. We would also like to apply functional groups with NO-releasing properties which are able to protect gastric mucosa from negative effects caused by COX inhibition.

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Poster communications

Poster communication 1

Correlation of polyphenolic content with radical-scavenging capacity and anthelmintic effects of Rubus ulmifolius (Rosaceae) against gastrointestinal nematodes from sheep

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Abstract

Phenolic content, antioxidant and anthelmintic activities of herbal extracts are of particular interest to drug industry; plant extracts with significant anthelmintic activity have the potential to be used as alternatives to conventional chemical drugs. In the present study, *Rubus ulmifolius* fruit extracts obtained using solvents of increasing polarity (water, methanol, chloroform and hexane) were examined for their antioxidant and anthelmintic activities in correlation with their polyphenolic content. *In vitro* antioxidant activity of all extracts was carried out using free radical-scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and2,2'-azinobis-(3-ethilenebenzotiazolin)-6-sulfonic acid (ABTS) radical cation. *In vitro* anthelmintic activities were investigated on the egg and adult worms of *Haemonchus contortus* from sheep in comparison to albendazole.

Total polyphenol content of *R. ulmifolius* was higher in more polar extract, ranging from 64.5 in aqueous extract to 1.57 mg gallic acid equivalents per gram of dry weight (GAE/g DW) in hexanic extract. Likewise, highest amounts of flavonoids and condensed tannins were found in aqueous extract (28.06 mg QE/g and 7.42 mg CE/g DW, respectively) compared to hexanic extract (0.71 mg QE/g and 0.29 mg CE/g DW, respectively) (p<0.05). Both DPPH and ABTS antioxidant assays showed that all tested extracts possess free radical scavenging activity, while the inhibitory concentration 50% (IC_{50}) range values were similar for both assays (2.13–45.54 µg/mL and 1.2–43.82 µg/mL, respectively).

All plant extracts showed ovicidal activity at all tested concentrations. Fruit methanolic (IC_{50}= 2.76 mg/mL) and aqueous (IC_{50}= 2.08 mg/mL) extracts showed higher inhibitory effects than chloroformic (IC_{50}= 7.62 mg/mL) and hexanic (IC_{50}= 12.93 mg/mL) extracts on egg hatching (p<0.05). There was a significant correlation of total polyphenol, flavonoids and tannins content with scavenging of either DPPH (r = 0.722, 0.764 and 0.752, p< 0.01, respectively), ABTS radicals (r = 0.893, 0.765 and 0.722, p< 0.01, respectively) and with inhibition of egg hatching (r = 0.874, 0.883 and 0.862, p< 0.01, respectively). Highest inhibition of motility (100 %) of worms was observed 8 h post-exposure in aqueous and methanolic extract at 8 mg/mL.

To our knowledge, these results depict for the first time that *R. ulmifolius* possesses *in vitro* anthelmintic properties.

Keywords: *Rubus ulmifolius;* Antioxidant; Anthelmintic; *Haemonchus contortus;* Egg hatching; Adult mortality

Poster communication 2

Structural Systems Biology to Elucidating Targetable Inescapable Hubs

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Rerouting resistance is a novel concept we proposed after noting cross-family subtleties during the characterization of proteins from primate (e.g. HIV-1) and non-primate (e.g. FIV) immunodeficiency viruses, which must hijack the host machinery for successful replication. Whereas the virus necessarily utilizes host cell machinery to replicate, it appears that the critical dependency lies at the pathway level and we hypothesize that a new kind of viral resistance will emerge, 'rerouting-resistance,' whereby the virus, taking advantage of cellular redundancies, can exploit its rich mutational repertoire to utilize unrestricted, functionally equivalent, cellular interactions critical for replication [1, 2]. We propose that specific protein-protein interactions matter less than the targeted cellular pathways, and that blocking one specific protein-protein interaction will be circumvented by available functionally equivalent alternatives. Therefore, efforts to develop drugs based on the knowledge of specific protein-protein interactions may be in vain unless a more holistic understanding of a given pathway interactome, including potential rerouting landscapes, is adopted. This requires the characterization of molecular nuances employed by the 'fitness' protein variants captured in various modules of a given cellular pathway. Mapping of rerouting landscapes uses classical structural and multi-omics tools to 1) identify the genetic sequence-determinants affording fitness and flexibility to mutant proteins, 2) characterize the *molecular buffering* mechanisms of these fitness *sequences* affording the acquisition of functional states, and 3) define the *mechanistic* basis of structural promiscuity affording fitness and flexibility in rerouting using structural biology approaches.

Embracing the *rerouting resistance* concept will open up new horizons for a novel research trend that places detailed descriptions of individual protein-protein interactions into a global picture. Such a global view considers the relevance and exchangeability of the specific interactions with respect to the background of the entire network of pathways, importantly including a description of redundancies and alternatives in pathway routes. Ultimately, focusing on mapping rerouting landscapes should eventually emphasize inescapable pathway *nodes* amongst the many individual replaceable pathway components. ***Novel intervention strategies need to target inescapable pathway nodes rather than individual replaceable components.***

Pathway targeting is not an innovation of parasitic pathogens, but rather a common biologic principle meaning that the exploration of the pathway rerouting phenomena will have wide applicability for normal and diseased cellular states including cancer.

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Poster communication 3

A multitarget approach for the *in silico* identification of bioactive compounds

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Almost twenty years ago, Melchiorre and his collaborators studied the pharmacological profiles of some small organic molecules against different receptors, thus demonstrating a multitarget mechanism of action of purposely designed compounds [1]. Since then, the multitarget drug discovery and polypharmacology research fields have grown sharply, with the aim to potentially overcome some of the major limitations of the classic "one target, one drug" paradigm. In particular, state-of-the-art computational approaches offer the possibility to predict the activity profile of ligands to a set of targets, thereby anticipating potential selectivity or discovering desired multitarget activities in the iterative design and optimization steps typical of a preclinical drug discovery project [2].

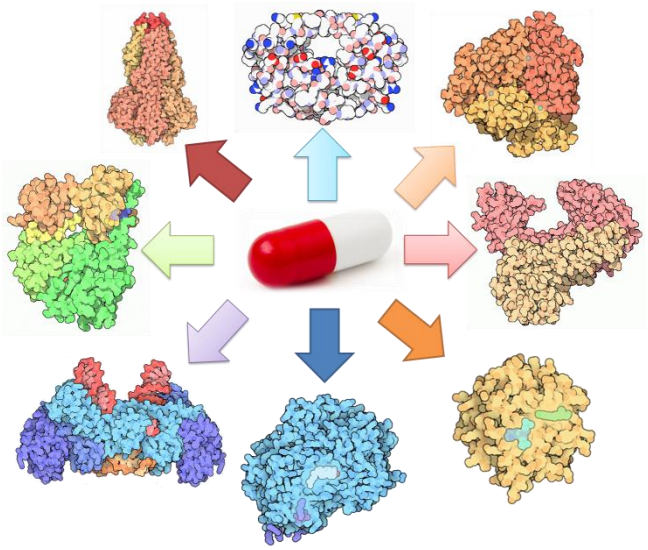

Figure 1: A schematic representation of the multitarget paradigm.

For this purpose, we created a chemoinformatics database by including natural and synthetic compounds in order to apply a virtual screening approach against different targets related to relevant diseases.

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Poster communication 4

Polyamines, acidic retinoids, psoralens and taepeenin D analogs, conjugates and hybrids: natural products with potential multi-target activity

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Our research group, for over 20 years, has been involved in the synthesis and/or chemical modification of natural products with biological-pharmaceutical interest, such as polyamines, acidic retinoids, psoralens, and, very recently, taepeenin D.

Polyamines (PAs), including spermine, spermidine, and putrescine, are present in the vast majority of cells of living organisms. They play important roles in the synthesis of proteins, cell division, differentiation etc. Moreover it is well established in the literature that naturally occurring PAs or their conjugates with other biomolecules are endowed with interesting biological properties/activities.[1] For this reason, we have developed several synthetic methodologies for the total synthesis of a plethora of selectively protected natural and synthetic polyamines, suitable for conjugation to other biologically active molecules, aiming to improve their biological properties.[2a,b]

On the other hand, retinoids, a large family of natural and synthetic compounds structurally related to vitamin A, play important roles in a variety of biological functions and have been applied successfully to the management of severe skin disorders and more recently to cancer prevention and therapy. The synthetic analogs of *all-trans*-retinoic acid (ATRA) 13-*cis*-retinoic acid (isotretinoin) and acitretin (ACI) are presently regarded as the drugs of choice for the treatment of several dermatological disorders. Quite recently, we have presented syntheses, theoretical characterization and results from a study of the antiproliferative activity of acitretin-type retinoids with changes in the lipophilic part of the molecule.[3]

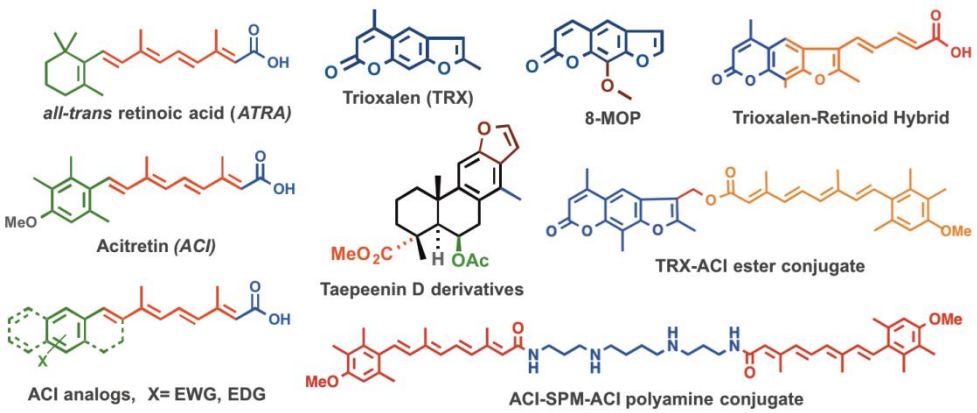

Figure 1: Chemical structures of several natural product derivatives described herein.

Furthemore psoralens, like 4,5',8-trimethylpsoralen (trioxsalen, TRX) and 8-methoxypsoralen (8-MOP), are commonly used in combination with ultraviolet A light radiation for the systemic treatment by photochemotherapy (PUVA) of skin hyperproliferative diseases like psoriasis. In connection with our research on ACI, we have synthesized a series of C4'-modified trioxsalen derivatives which were successfully used for the preparation of a variety of trioxsalen conjugates with ACI of either the PA or the ester type as well as trioxsalen–acitretin hybrids.[4]

Finally, very recently, we started a new collaboration with Dr. Pitsinos' research team from NCSR "Demokritos", aiming to the development of new Hedgehog (Hh) inhibitors. Our attention was caught by the natural product Taepeenin D. This cassane-type diterpenoid was found to be a low micromolar inhibitor of the Hh pathway, while exhibiting selective cytotoxicity against cancer cell lines.[5] Accordingly, we explored the effect of structure modifications on the biological activity a series of Taepeenin D derivatives. By exploiting abietic acid, as a readily available chiral template, we were able to prepare 14-desmethyl- and 6-deoxy-14-desmethyl-Taepeenin D derivatives.

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Poster communication 5

Discovery and development of new anti-invasive agents, an unmet clinical need

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The sequelae of invasion and metastasis are responsible for 90% of cancer-related mortality. Unfortunately, no effective inhibitors of these events are available in the clinic.[1] In response to this unmet need, our laboratories have embarked on a quest for small-molecule tools and leads for the study and pharmacological manipulation of local invasion. Our efforts in this area will be presented in the current communication. The underlying therapeutic strategy aims at reducing mortality due to late metastasis in cancer patients.

In one project, for example, a screen using a highly relevant invasion assay (3D confronting culture of precultured chick heart fragments (PHF) and aggregates of breast carcinoma cells, Figure 1) afforded low micromolar hits.[2,3] A chemical optimization program yielded analogs with nanomolar potency. No red flags emerged during extensive eADME-Tox, PK and MTD profiling. One compound was evaluated in vivo and increased survival time in an artificial metastasis model.

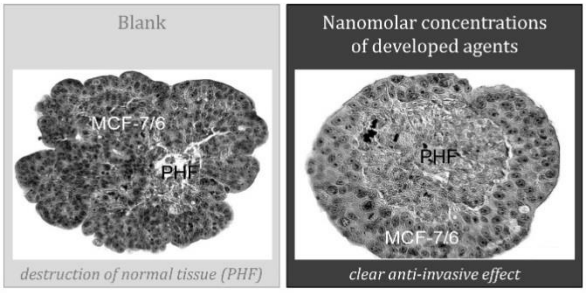

Figure 1: Efficacy of developed agents in a highly relevant invasion assay (3D Confronting culture).[2]

The molecular mechanism of action of the compounds remains unknown, but has been distinguished from that of known anti-invasive/antimetastatic molecules in development. Current efforts are focusing on the further establishment of efficacy in more complex animal models and on the identification of the MMoA of our molecules.

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Poster communication 6

Biological targets and rationale development of their modulators

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Our medicinal chemistry research is focused on rationale identification and synthesis of appropriate organic compounds possessing the ability to modulate an activity of selected biological targets. We are performing ligand / target interaction analyses, SBDD (*Structure Based Drug Design*), modelling and virtual screening leading to development (computer predictions / organic synthesis / bioactivity evaluation / optimization) of target inhibitors e.g. VEGFR2,[1-4] Mer, Axl, Clk kinases (potential oncology therapeutics) and modulators of SMO GPCR (a part of the Hedgehog pathway involved in stem cell biology useful for development of anticancer or regenerative medicine drugs). Within our research we identified an unknown type of ligand supported tyrosine kinase conformation possessing special DFG-in/out arrangement with an opened A-loop.[2,5] Recently we have developed several nM VEGFR2 and CLK-1 kinase inhibitors based on N-aryloxazol-2-amine (Fig. 1) and chromenopyridinone skeletons. The binding poses for our inhibitors were predicted and the biological activities determined by radiometric enzymatic assay: IC_{50}: 13 - 87 nM (VEGFR2)[3,4] and IC_{50}: 20 - 80 nM (CLK1).[5] Moreover we are preparing some predicted ligands dedicated to identification of novel kinase inhibitors by *In situ Click* chemistry.

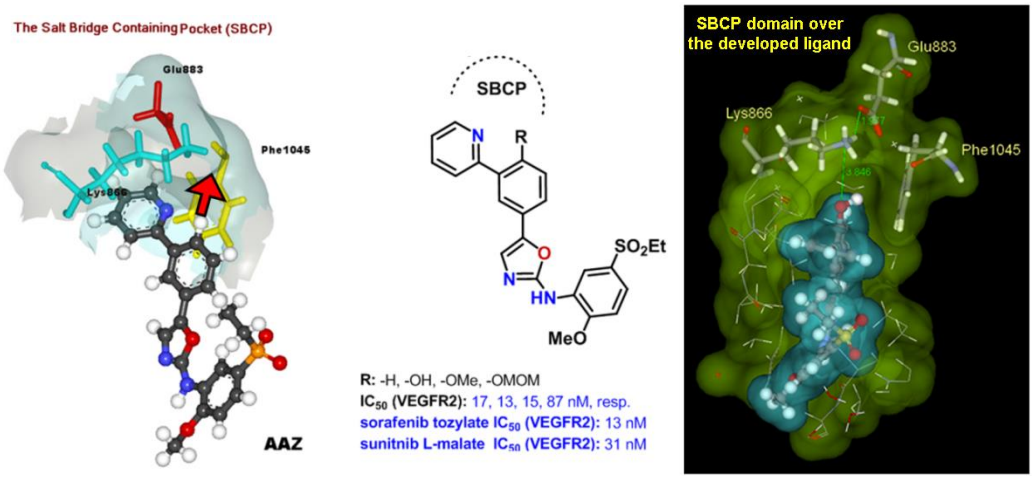

Figure 1: Some of our aminooxazole inhibitors designed to support assembly of a special DFG-in/out kinase conformation possessing an SBCP domain.

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Poster communication 7

Novel tacrine-resveratrol hybrids as multi-target-directed ligands to combat Alzheimer's disease

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Multi-target drug discovery is one of the hottest topics and most active fields in the search for new drugs against Alzheimer's disease (AD).[1] This is because innovative multi-target-directed ligands (MTDLs) could more adequately address the complexity of this pathological condition, for which we still lack suitable therapies. In a continuation of our efforts aimed at achieving enhanced therapeutic efficiency with respect to single-target drugs, we developed a new series of anti-AD MTDLs.

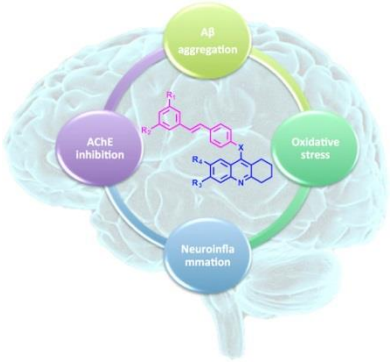

Figure 1: MTDL profile of tacrine-resveratrol hybrids.

The compounds were designed by combining the structural features of the cholinesterase inhibitor drug tacrine with resveratrol (Fig. 1), which is known for its purported antioxidant and anti-inflammatory activities in the brain.[2] The most interesting hybrid compounds inhibited human acetylcholinesterase at micromolar concentrations and effectively in vitro modulated Aβ self-aggregation. In addition, they showed intriguing anti-inflammatory and immuno-modulatory properties in neuronal and glial cell-based AD models. Importantly, the MTDL profile is accompanied by high predicted blood-brain barrier permeability, and low cytotoxicity both in HepG2 cells and primary neurons.

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Poster communication 8

Crassiflorone-derived multi-target-directed ligands against trypanosomiases

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Single chemical entities modulating multiple targets have emerged as more efficacious and tolerable than the available drugs to combat neglected tropical diseases.[1] In this context, we recently reported on the identification of a series of quinone-coumarin hybrids directed against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and trypanothione reductase (TR).[2] These enzymes belong to metabolic pathways that are vital to *Trypanosoma brucei* and *T. cruzi*, and have been validated as antitrypanosomal drug targets. In the search of novel dual-targeted inhibitors of these enzymes, we envisaged that crassiflorone (Fig. 1), a natural compound isolated from the African ebony *Diospyros crassiflora* with antimycobacterial and antigonorrhoeal activities,[3] might represent a suitable starting point. In addition to the intrinsic multi-target mechanism of action of a natural product, crassiflorone appeared a promising dual GAPDH/TR inhibitor, thanks to its pentacyclic core consisting of a fused furocoumarin/naphthoquinone scaffold.

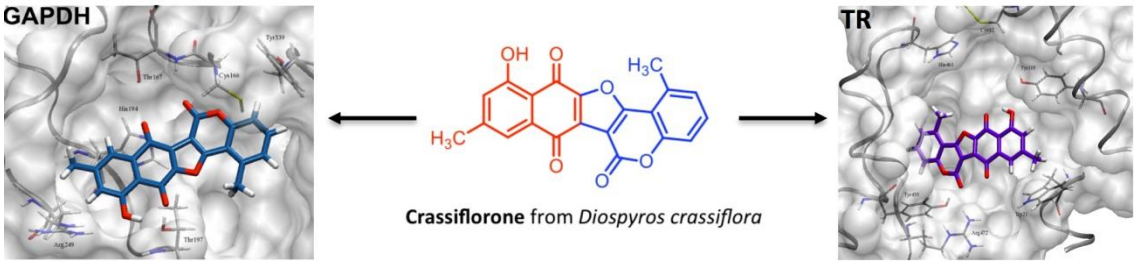

Figure 1: Docking of crassiflorone at *Tb*GAPDH and *Tb*TR binding sites.

Through purposely-addressed docking studies, we verified that crassiflorone can bind at both enzyme active sites, thus confirming the design rationale. On this basis, a small set of crassiflorone derivatives have been synthesized and their dual-target antitrypanosomal profile characterized both in enzymatic assays and in in vitro parasite cultures.

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Poster communication 9

From bitopic inhibitors to multitarget drugs to fight against Alzheimer's disease

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Dementia is one of the main causes of the disease burden in developed regions. According to the World Health Organization (WHO), it will become the world's second leading cause of death by the middle of the century, overtaking cancer. This will have a dramatic impact on medical care, and have important social and economic implications, unless more effective preventive procedures or treatments become available. Alzheimer's disease (AD) is the most common cause of dementia, accounting for approximately 50–75% of all dementias worldwide, followed by vascular dementia, mixed dementia, and Lewy body dementia.

Research to develop effective treatments for AD has evolved over the last decade. Anti-AD drug research started with the traditional single-target approach (one-to-one) where one selective drug acts on one specific target, for example, tacrine or donepezil as selective drugs for AChE inhibition. The next step was the development of bitopic drugs, in which one drug acts simultaneously at two binding sites of the same target. This strategy, where a unique drug takes advantage of interacting with one enzyme (AChE) at two different blocking sites and producing two different biological activities, may be considered the basis of the subsequent multitarget drug approach (one-to-two) where one drug acts on two or more targets (Figure 1).

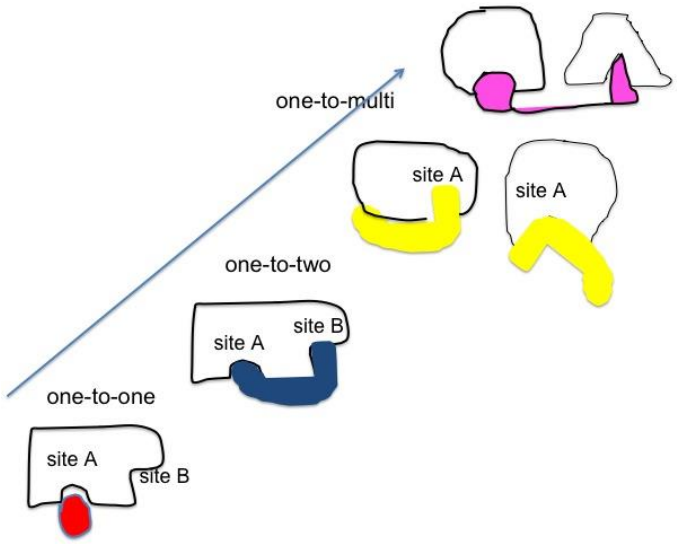

Figure 1. Progress in the strategies to develop anti-Alzheimer drugs.

Herein, we will explore the journey of our research group from a bitopic inhibitor strategy to multitarget drugs for the future treatment of AD.

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Poster communication 10

Proteomic tools to predict nanodrug targeting and uptake

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Nanotechnologies hold enormous potential to revolutionize the field of nanomedicine through applications in nanovaccines, diagnostic imaging tools or targeted drug delivery. What are the essential properties to determine if a new synthesized nanomaterial for medicine (NMM) will function efficiently under safety conditions? How to systematically evaluate which properties from a new NMM could improve the performance from previous formulation? Our research group has been applying proteomics based methodologies for nanotoxicology and nanosafety that can be implemented to answer those questions. Here, we will present two methods that could address: i) if a nanoparticle can reach the target cell; ii) if a nanoparticle can be uptake by the target cell. The final goal of this project is to integrate several methods based in lab-in-a chip and proteomic analysis that could offer a platform to assist the safe by design principle for new engineered NMs with application for biomedicine.

The development of those nanoparticles (NP)s and nanomaterials (NM)s with specific properties aims to obtain specific effects after exposure but on the other side additional secondary or harmful effects should be evaluated. The balance between desirable effects (biocompatibility and desired activity) and he possible adverse effects (cytotoxicology, bioaccumulation) should be evaluated at an early stage. Therefore it is required to develop an integrated platform for prediction functionality, diagnosis and at the same time assessment of possible harmful effects that could be systematically applied any new engineered nanomaterials for biomedical applications. Quantitative proteomics and surface proteomic analysis can offer high-throughput analysis at high resolution for many possible formulations at a reduced time compared to classical in vitro assays .

Poster communication 11

Investigating the constitutive activity of Ghrelin receptor by molecular modeling

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Ghrelin (GHR) system is involved in a large number of physiological and pathological problems, making this system an appealing pharmacological target. In particular, it has a pivotal role in the regulation of food intake, energy homeostasis, and alcohol/drug reward. The related pleiotropic peptide, the GHR, carry out its role through the interaction with the growth hormone secretagogue receptor isoform 1a (GHS-R1a) [1–3]. The GHS-R1a is a seven trans-membrane G-Protein Coupled receptors member of Class A subfamily, characterized by a unique constitutive activity (up to 50% of the total activity is ligand-independent) [4]. Despite the importance of this system in terms of pharmaceutical R&D and medical benefit, the molecular mechanism driving the GHS-R1a basal activity is not fully clarified yet. In this work, after the creation of a novel, accurately refined homology model, Classical Molecular Dynamics in combination with Metadynamics were employed to get insight into GHS-R1a receptor structural dynamics[5]. Specifically, several conformational changes have been highlighted and fully characterized from the energetic point of view, therefore elucidating the molecular events governing the ligand-independent activity. In agreement with the speculation based on experimental data, peculiar residues located in the GHS-R1a binding pocket (Trp276, His280 and Arg283) have been identified as fundamental elements affecting the process. In detail, it has been proposed that the Arg283 is able to stabilize the basal activation, affecting the Trp276 position through its interaction with His280. This is of great importance considering that an improvement in the knowledge of molecular processes regulating the GHS-R1a function may help the rational design of new drugs for the treatment of a large number of pathologies.

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Poster communication 12

Drug-conjugation emerging properties: molecular insights

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Drug-conjugation is known to profoundly affect not only pharmacokinetic (PK) profile, but also the drug activities on cellular targets. Specifically, drug conjugation offers a successful way to customize drug properties without modifying the structure of the pharmacophore. Different kinds of conjugates are contemplated in the pharmaceutical context, such as proteins, liposomes, polymers, etc. A specific class of conjugates is usually selected based on both the characteristics of the pharmacophore and the therapy requirements, for example to optimize the PK or pharmacodynamic (PD) profiles, to preserve the drug and to offer a controlled and targeted delivery. The conjugated molecule interacting with tissues, organelles and macromolecules establishes a series of biological effects. Therefore, final drug properties could result from the acquisition of new physicochemical, structural and functional characteristics, influencing solubility, aggregation tendency, affinity for the target, pharmacophore shielding, recognition by body “sentinels” (enzymes, immune system), etc. For all these reasons, an appropriate prediction and understanding of the tail-derived properties is necessary to produce a successful conjugation.

In the present study, we investigated the effect of the linkage at different levels through computational simulations, in order to evaluate which are the conjugation-sensible aspects of the drug “path” before activating an intracellular target. In particular, we analyzed how the conjugation with phospholipids (PLs) or polyethylene glycols (PEGs) could influence not only the compounds behaviour in solution (e.g. aggregation tendency) and the cellular internalization, but also the affinity for the target and its activation. Large time and space scales are needed for this type of studies, therefore we used both atomistic and coarse grain approaches. The methods were chosen according to the nature (in particular the staticity-dinamicity) of the event of interest (e.g. Jarzynski equality to analyze the membrane crossing and umbrella sampling to evaluate the interaction between ligands and binding site).

Poster communication 13

A Comprehensive Description of the Homo and Heterodimerization Mechanism of the Chemokine Receptors CCR5 and CXCR4

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Signal transduction across cellular membranes is controlled by G protein coupled receptors (GPCRs). It is widely accepted that members of the GPCR family self-assemble as dimers or higher-order structures being functional units in the plasma membrane. The chemokines receptors are GPCRs implicated in a wide range of physiological and non-physiological cell processes. These receptors represent prime targets for therapeutic intervention in a wide spectrum of inflammatory and autoimmune diseases, heart diseases, cancer and HIV. The CXCR4 and CCR5 receptors are two of the manly studied playing crucial roles in different pathologies. In this scenario the use of computational techniques able to describe complex biological processes such as protein dimerization acquires a great importance. Combining coarse-grained (CG) molecular dynamics and well-tempered metadynamics (MetaD) we are able to describe the mechanism of dimer formation, capturing multiple association and dissociation events allowing to compute a detailed free energy landscape of the process

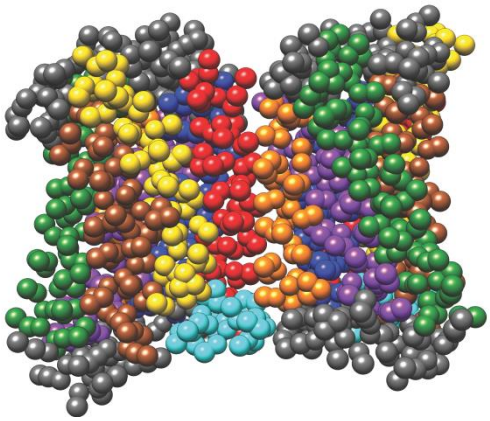

Figure 1: A representative snapshot of the coarse-grained simulation

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Poster communication 14

Design of acridine derivatives with potential antiproliferative activity based on multi-target action

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Acridine derivatives posses antiproliferative activity due to their main mechanism of action – DNA intercalation and subsequent inhibition of DNA related enzymes, such as topoisomerases and telomerase. It was also found that some acridine derivatives inhibit various kinases, such as Src, MEK and VEGFR-2, which contributes to their antiproliferative activity.[1,2] The aim of this study was design of novel acridine derivatives with potential antiproliferative activity, based on multi-target action – DNA intercalation, as well as inhibition of Src, MEK or VEGFR-2 kinases. These compounds contain aminoacids (L-glycine, L- and D-phenylalanine, L-histidine and L-asparagine) or corresponding dipeptides in C9 side chain (Figure 1). Molecular docking studies were performed in AutoDock Vina program.

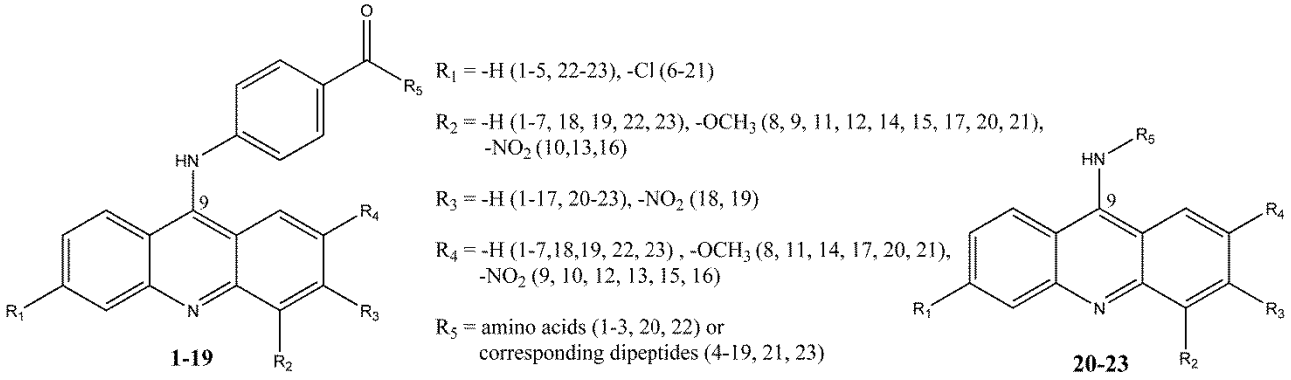

Figure 1: Chemical structures of compounds tested in this study (**1**-**23**).

All tested compounds bind to DNA similarly to amsacrine, which was used as standard, apart from derivatives **3**, **4**, **5** and **6**. Derivatives with lowest binding energies which form key binding interactions with MEK were **4**, **6**, **9**, **10**, **11**, **12**, **13**, **15**, **16**, **18** and **19**, with VEGFR-2 were **8**, **11** and **16**, whereas with Src were **4** and **6**. Significant antiproliferative activity and multi-target action could be expected from these compounds.

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Poster communication 15

Targeting the PI3K/mTOR pathway as an antitumor strategy: 3D-QSAR study and design of dual PI3K/mTOR inhibitors

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Deregulation of the phosphatidylinositol-3-kinase (PI3K)/ mammalian target of rapamycin (mTOR) signaling pathway is one of the most common abnormalities found in various types of tumors and therefore represents the potential target place for new antineoplastic drugs. A dataset consisting of 85 dual PI3K/mTOR inhibitors was collected from literature and divided into two groups based on the structural analogy. In order to obtain information about the most important structural determinants that dictate dual PI3K/mTOR inhibitory activity, 3D-QSAR study was applied on each group, resulting in four PLS models. Internal and external validation parameters demonstrated high quality and good predictive ability of each 3D-QSAR model (Q^2=0.72, R^2_{pred}=0.93; Q^2=0.81, R^2_{pred} =0.88 for 3D-QSAR (mTOR) models and Q^2=0.79, R^2_{pred}=0.93; Q^2=0.79, R^2_{pred}=0.94 for 3D-QSAR (PI3K) models).

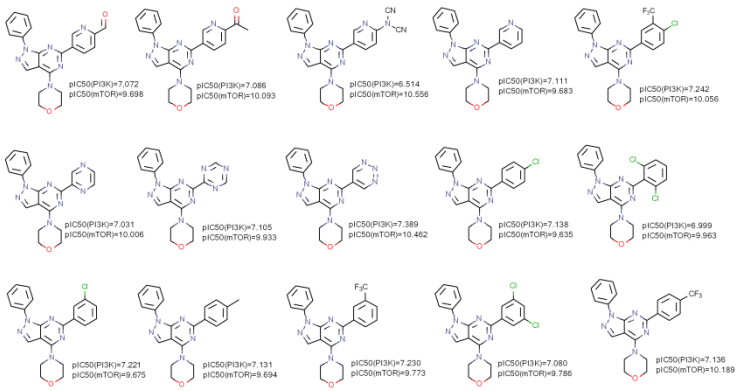

Figure 1: Structures of selected designed PI3K/mTOR inhibitors

After the analysis of 3D-pharmacophoric features, new compounds were designed and those with high activity on both enzymes were further screened for ADMET properties. Finally, 15 new PI3K/mTOR inhibitors with better activity and improved ADMET properties compared to the starting compounds were selected as novel drug candidates with enhanced anticancer activity.

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Poster communication 16

Benzopyrane as a valid scaffold for the development of multi-target ligands for neurodegenerative diseases

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Neurodegenerative diseases are characterized by a progressive nervous system dysfunction, and a consequently deterioration in the cognitive function. These disorders, often associated with atrophy of the affected central or peripheral structures of the nervous system, include Alzheimer's (AD), Parkinson's (PD), among others. There is now a comprehensive understanding that neurodegenerative diseases share several common multifactorial processes that could contribute to neuronal death. Consequently, drug discovery programs based on the concept one drug-one target have failed to provide novel and effective drugs to cure or ameliorate the course of the disease.[1] To overpass these hurdles, multi-target-directed-ligands (MTDLs) have been increasingly exploited for hitting different biological targets[1], for instance acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), monoamine oxidase (MAO) or some adenosine receptor (AR) subtypes.

Privileged structures are important tools in the design of new ligands contributing for the on-going changing of drug discovery landscape. In this framework, chromones are by now considered important privileged structures for the design of chemical libraries directed at a broad spectrum of targets.[2-4] Accordingly, a project encompassing the discovery and development of NCEs with the ability to interact with several targets of neurodegenerative diseases has been developed. Herein, it is presented the data so far obtained regarding the synthesis of chromone based libraries, along with their biological evaluation toward ARs, MAO-B and AChE/BuChE.

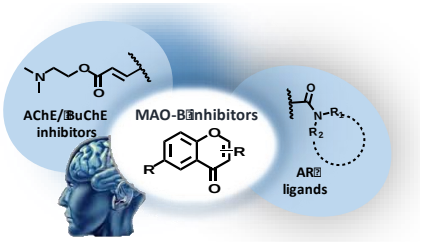

Figure 1: Benzopyrane as a valid scaffold for the development of multi-target ligands

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Poster communication 17

Targeting the human 20S proteasome through a computational-based drug discovery approach

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The ubiquitin proteasome system (UPS) is a nonlysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle regulation and apoptosis, but also in inflammatory and immune responses, carcinogenesis, among others.[1] Usually in protein homeostasis the defective proteins are ubiquitinated and are proteolysed into short peptides by the proteasome.[1] Proteasome substrates include, for example, signalling molecules, tumour suppressors, cell cycle regulators and transcription factors.[2] Proteasome inhibition results in an interruption of the degradation of these substrates, leading to activation of apoptotic pathways and, eventually, cell death.[1] Rapidly growing cells, such as cancer cells, are particularly susceptible to proteasome inhibition.[3]

This work relies on a computational based drug discovery campaign to discover alternative new, selective (and more effective) small molecule reversible proteasome inhibitors that can overcome the severe adverse drug reactions demonstrated by in use drugs. The efforts to discover new anticancer drugs described here combine different computer-aided drug design methodologies like pharmacophore modeling, molecular docking and structure-based virtual screening to identify potential hit compounds. These *in silico* methodologies illustrate the importance of small-molecule key features in proteasome activity and can also help in the discovery process of potential novel medicines with completely new chemotypes.

Acknowledgements: We thank the Fundação para a Ciência e a Tecnologia for financial support (SFRH/BD/104441/2014, PTDC/QEQ-MED/7042/2014, and UID/DTP/04138/2013).

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Poster communication 18

D2AAK1 as a potential multi-target antipsychotic

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The modern approach to drug design and discovery for the treatment of complex diseases, like neurodegenerative diseases, cancer and many psychiatric disorders, involves searching for medicinal substances which fulfil criteria of several pharmacophores, instead of acting on a single molecular target. Indeed, in complex psychiatric illnesses, including schizophrenia, selective single-target drugs have been to a great extent a failure. The pharmacological profile of clozapine reflects the molecular pathogenesis of schizophrenia, which involves cross-talk of many neurotransmitter systems (especially dopaminergic, serotonergic, adrenergic and glutamatergic). The new paradigm in drug design and discovery is to search for compounds which modulate the activity of several molecular targets simultaneously. To achieve this, it is necessary to identify structural features that link important classes of drug targets, which will enable the design of drugs with the desired selectivity profiles.

We identified a novel dopamine D_2 receptor antagonist, D2AAK1, with Ki of 58 nM using structure-based virtual screening.[1] D2AAK1 possesses additional nanomolar or low micromolar affinity to D_1, D_3, 5-HT_{1A} and 5-HT_{2A} receptors, making it an ideal candidate for a multi-target drug.[2] Here we present homology modeling, molecular docking and molecular dynamics of D2AAK1 and its molecular targets and animal studies of D2AAK1 as a potential antipsychotic. The main contact of D2AAK1 and all the receptors studied is the electrostatic interaction between the protonable nitrogen atom of the ligand and the conserved Asp(3.32) as typical for orthosteric ligands of aminergic GPCRs. We confirmed antagonistic/partial agonistic properties of D2AAK1 towards all the receptors in *in vitro* essays and in *in silico* studies as the ligand stabilizes the ionic lock interaction. We also demonstrated neuroleptic, anxiolytic and, importantly, procognitive properties of D2AAK1 in mouse models.

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Poster communication 19

From acetylcholinesterase inhibitors to multitarget compounds for the treatment of Alzheimer's disease

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Alzheimer's disease (AD) is a multifactorial disorder and it apparently involves several different etiopathogenetic mechanisms. Up-to-date, there are no curative treatments or effective disease modifying therapies for AD. On the other hand many aspects of AD are currently debated or even unknown. Current efforts in the development of novel drugs aimed against AD are represented by the so-called Multi-Target-Directed Ligands (MTDLs), the therapeutic strategy followed not only in the AD research but also in other diseases.[1] MTDLs combine drugs action at different levels of the neurotoxic cascade. Modulating a multiplicity of interconnected targets with an MTDL is an asset in treating a complex disorder of the elderly. Within our contribution, novel trends in designing and development of novel MTDLs as potential anti-AD drugs will be presented.

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Poster communication 20

tert-amylphenoxy derivatives of 4-methyl- and homo-piperidine ligands of histamine H3 receptor as acetyl - and butyrylcholinesterase inhibitors

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder in the elderly. The loss of cognitive functions in AD patients is mainly connected with cholinergic neurotransmission decline in the brain. Currently available treatments for AD (donepezil, rivastigmine, galantamine and memantine) are symptomatic and do not reverse, stop, or even slow the progression of the disease. Due to the complex pathology of AD multitarget-directed ligands (MTDLs) are suggested to be able to produce the desired efficacy.[1] One of the multitarget approach is to combine cholinesterases inhibition with additional properties, e.g. histamine H_3 receptor (H_3R) antagonism. Blockade of histamine H_3R with selective antagonists/inverse agonists can increase the release of neurotransmitters such as acetylcholine, dopamine or serotonin. Effectiveness of some H_3R antagonists/inverse agonists in patients with AD have been investigated in clinical studies.[2]

In this work we selected by application of virtual screening the potential acetylcholinesterase inhibitors from the group of histamine H_3R ligands - *tert*-amylphenoxy derivatives of 4-methyl- and homo-piperidine (hH_3R K_i: 21-308 nM)[3]. The ChemScore function and analysis of binding mode enabled to choose hits which were evaluated *in vitro* for acetyl- and butyrylcholinesterase inhibition. The target compounds showed cholinesterase inhibitory activity in a low micromolar and submicromolar range. The most potent in this group was 1-(7-(4-*tert*-amylphenoxy)heptyl)homopiperidine inhibiting the both enzymes (*Ee*AChE IC_{50} = 4.83 µM and *Eq*BuChE IC_{50} = 0.73 µM). The presented studies let us find multitarget ligands directed towards histamine H_3R and cholinesterases.

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Poster communication 21

Crossing the interface between inorganic and organic chemistry, biology and pharmacology to generate innovative chemotherapeutics beyond those currently in use

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Despite highly toxic regimes, massive public education and much research worldwide, cancer remains one of the most deadly diseases known to man. Platinum drugs are amongst the most wide used anti-cancer agents.[1,2] Their anti-cancer activity is attributed to their ability to bind DNA, leading ultimately to apoptosis. Despite their success, the clinical utility of platinum drugs is limited due to acquire/intrinsic drug resistance and/or systemic toxic side effects. There remains an urgent need therefore to develop innovative therapeutics beyond those currently in use. Inspired by (i) the clinical success of platinum drugs, (ii) the more recent clinical success of small molecule inhibitors of histone deacetylase (HDAC) enzymes as anti-cancer agents and (iii) extensive literature reports indicating the therapeutic potential of complexes incorporating metals other than platinum, through rational drug design we have advanced a new class of multi-functional platinum[3-5] and non-platinum chemotypes with a mechanism of action and cytotoxicity profile different to classical platinum drugs. A summary of our results to date will be presented.

*This material is based upon works supported by the Science Foundation Ireland under Grant No. [11/RFP.1/CHS/3094] and [12/TIDA/B2384], the Higher Education Autority (*Bioanalysis and Therapeutics BioAT*) and an RCSI Apjohn scholarship. We thank colleagues in COST CM1105 for fruitful collaborations and COST CA15135 for financial support.*

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Poster communication 22

Heterocovariance based metabolomics as a powerful tool accelerating bioactive natural product discovery

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Natural products possess significant and well-documented biological properties representing the bioactive chemodiversity hot-spot in the chemical space, however the great majority of existing compounds remains still unexplored. Traditional isolation and scale-up procedures are inefficient and often become the bottleneck in natural products dereplication in order not to re-identify known compounds that are responsible for the activity.

We demonstrate in this work that, spectral data reflecting concentration differences of the components of an extract can correlate statistically with measurable dose-dependent properties such as enzyme inhibition, on the basis of a Heterocovariance approach, identifying the bioactive components prior to purification. A key point is to create a concentration variance of the components to be correlated with bioactivity by a carefully planned fractionation of the plant material. In this aspect, medicinal plants were extracted and fractionated using the Centrifugal Partition Chromatography technique (CPC). The NMR and MS spectra of specific number of fractions were recorded as well as their ability to inhibit tyrosinase enzyme. Activity was correlated with series of NMR and mass peaks. Compounds were identified from the statistically mined out spectroscopical data and the procedure was validated by purifying and recording the spectra which were in perfect agreement with those deduced through the Heterocovarience analysis.

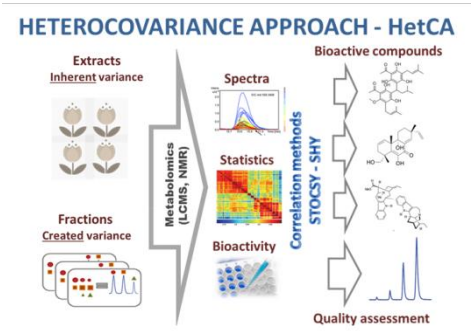

Figure 1: Heterocovarience plant metabolomics

In conclusion plant metabolomics provides a very powerful tool that can revolutionize natural products discovery. Inherent or carefully planned variance in concentration of plants' secondary metabolites as conveyed by NMR and MS spectra can be correlated with any dose-response property and unmask active constituents in the complex extract or fraction mixtures prior to any purification step. This highly innovative activity-based-metabolite-profiling can dramatically accelerate the discovery of active natural products challenging global biodiversity and chemodiversity.

Poster communication 23

In silico approaches to target multiple sequences of G-quadruplex DNA

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G-quadruplex structures (G4s) are non-canonical high-order topologies frequently present at crucial positions of the genome such as at telomeric ends and ribosomal DNA (rDNA), RNA, or gene promoter regions (e.g., *c-myc*, *bcl-2*, or *c-kit*) [1]. These G4 structures are involved in several physiological events. For instance, the presence of G4s at telomeres is fundamental to maintain the genome integrity, whereas in non-telomeric regions, such as oncogenic promoters, the formation of G4 structures can modulate gene transcription. In cancer cells, G4s represent an innovative antitumor target since it has widely demonstrated that the stabilization of G4s at the telomeric regions could indirectly inhibit the activity of telomerase overexpressed in 80–90% of tumor cells [2], while in promoter regions hinders the correct assembly of the transcriptional machinery, which alters the levels of gene expression.

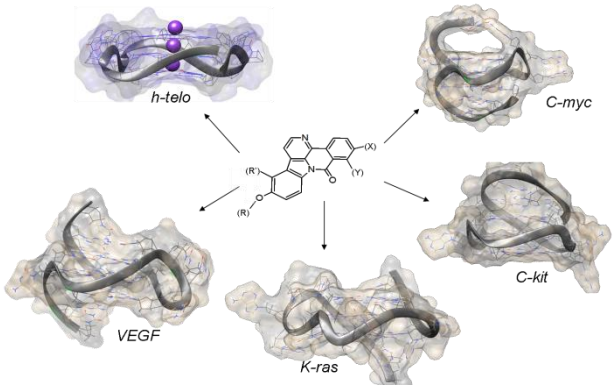

Figure 1: Schematic representation of the presenting work.

In this work, we present an extension of our previous Virtual Screening campaign [3] in which a novel naphthyridin-8-one derivative has been identified as a good G4 stabilizer of the *h-telo* sequences and, unexpectedly, also for the G4 of the *c-myc* sequence. Starting from these results, we have created an extensive focused library of naphtiridine-scaffolds derivatives and we have applied *in silico* approaches to multiple G4s of other oncogenic promoter sequences (*c-kit*, *k-ras* and *VEGF*) in order to identify more promising derivatives with multiple mechanism of action.

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Poster communication 24

Lipidic *anti*-β-amino alcohols as selective inhibitors of CK1ε

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Long chain amino alcohols from Nature have demonstrated significant activity as antitumor and cytotoxic drugs. As part of our interest in antiproliferative compounds, we have been involved in the synthesis and biological evaluation of diverse long chain amino alcohols.

A small and structure-biased library of enantiopure *anti*-β-amino alcohols was prepared in a straightforward manner by a simplified version of the Reetz protocol.[1] The antiproliferative activity testing against a panel of five human solid tumor cell lines gave GI_{50} values in the range 1-20 μM. A computational approach was designed to anticipate the molecular target(s) on the basis of the chemical structure and the antiproliferative activity. The methods pointed to kinases as plausible candidates. Experimental determination of the interaction with 456 kinases indicated that these *anti*-β-amino alcohols behave as selective CK1e inhibitors. Docking studies agreed with these findings.[2] The resulting lead, GSD0054 (Figure 1) was submitted to further mechanistic studies.

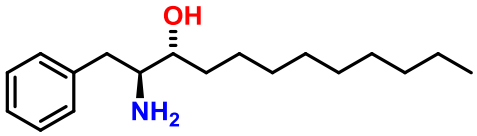

Figure 1: Structure of CK1ε selective inhibitor GSD0054.

Although GSD0054 behaved as selective CK1ε inhibitor in enzymatic assays, in this work we studied whether this effect was corroborated inside the cells. The effects of GSD0054 on protein expression and cell cycle disruption were studied in a panel of human solid tumor cell lines. We also performed molecular modeling studies using computational docking, against CK1δ and CK1ε, to explain and predict the mechanism of action of these compounds. In addition, the commercially available CK1ε inhibitors IC 261, PF-4800567 and PF-670462 were studied for comparison purposes.

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Poster communication 25

Identification of Novel PPARα and CB_1 Receptor Ligands As Antiobesity Drugs

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Cannabinoid (CB) and peroxisome proliferator-activated (PPAR) receptors are part of lipid signaling systems that play an essential role in regulating energy metabolism, inflammation and behavior [1,2]. N-acylethanolamines are a family of bioactive fatty acid derivatives that includes the endocannabinoid anandamide (N-arachidonoylethanolamide, AEA) and the satiety factor oleoylethanolamide (N-oleoylethanolamine, OEA) [1]. Whereas AEA is a CB_1 agonist, OEA is a PPARα agonist although both lipid mediators act on common signaling pathways and share metabolic enzymes related to their synthesis and degradation. Focused on energy metabolism and feeding behavior, CB_1 anatagonism and PPARα agonism have been reported to be a potential target for searching novel antiobesity drugs [1,2].

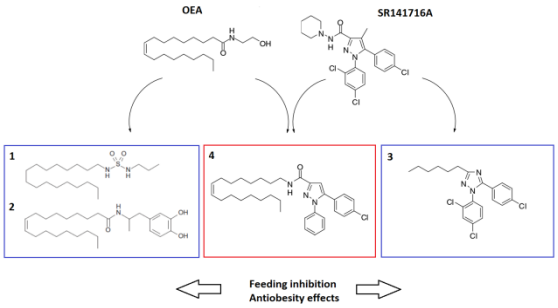

Figure 1: Structures of novel drugs with antiobesity effects based on OEA and SR141716A

In collaboration with Instituto de Química Médica (CSIC, Madrid, Spain) we have synthesized and evaluated several compounds *in vivo* and *in vitro* as CB_1 and PPARα ligands. Additionally, these novel compounds were evaluated as feeding suppressants and antiobesity drugs using rodent models with acute and repeated treatments. Among the most relevant anorexigenic compounds we found: N-octadecyl-N´-propyl-sulfamide (**1**) [3,4] and N-(1-(3,4-dihydroxyphenyl)propan-2-yl)oleamide (**2**) [5] based on OEA; 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole (**3**) [6,7] based on SR141716A; and N-(1-Oleyl)-5-(4-chlorophenyl)-1-phenyl-1H-pyrazole-3-carboxamide (**4**) [8] based on OEA and SR141716A. Searching novel ligands with a dual activity on CB_1 and PPARα appear as a promising strategy against obesity and other metabolic disorders.

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Poster communication 26

Xanthine derivatives as MAO-B inhibitors and adenosine receptor antagonists

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Parkinson's disease is a neurodegenerative disorder related primary to the progressive degeneration of dopaminergic neurons, especially in the substantia nigra of the brain. It causes a disturbance in the execution of arbitrary and involuntary movement sequences. Compounds with multiple activity that inhibit MAO-B and block A_1 and A_{2A} adenosine receptors are expected to show synergistic effects in the treatment of neurodegenerative diseases[1-3].

Previous research in the group of annelated xanthines confirmed their inhibitory activity toward A_{2A} and/or A_1 adenosine receptors. As a continuation of our study, three series of tricyclic xanthine derivatives substituted with a dopamine moiety were synthesized. The designed compounds were synthesized according to previously described procedures[4,5]. In addition, analogs lacking the third annelated ring were synthesized and investigated.

The synthesized compounds were tested in the radioligand binding assays for their affinity towards the four adenosine receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. All structures were tested on adenosine receptors expressed in Chinese hamster ovary (CHO) cells using membrane preparations. Additionally inhibitory activity towards monoamine oxidase type B (MAO-B) were determined by using commercially available human recombinant MAO-B expressed in baculovirus-infected insect cells, and the Amplex Red monoaminoxidase assay kit (Life Technologies). Drug-like properties (logP, logS, toxicity, drug score) of the obtained compounds were evaluated by using the OSIRIS program[6].

Potent dual-target-directed A_1/A_{2A} adenosine receptor antagonists which showed monoaminoxidase B inhibitory properties in the submicromolar range were identified in the current study.

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Poster communication 27

Identification of Lead Molecules Capable of the Simultaneous Agonism of the PPARγ and PPARα Subtypes for the Dual Management of Diabetes Mellitus and Dyslipidaemia.

Ellul Claire; Shoemake Claire

We report a rational drug design study aiming to identify novel lead molecules with the potential to act as agonists simultaneously at two Peroxisome Proliferator Activated Receptor (PPAR) receptor subtypes- specifically PPARγ and PPARα which could be successfully used in the dual management of Type 2 diabetes mellitus (through PPARγ modulation) and dyslipidaemia (through PPARα modulation). Two crystallographic depositions- pdb ID 3VN2 (Amano *et al.*, 2012) describing the holo telmisartan: PPARγ complex and pdb ID 2P54 (Sierra *et al.*, 2007) describing the holo GW590735: PPARα complex were used as templates. The affinity of the angiotensin receptor blocker telmisartan and that of the experimental fibrate GW590735 for their cognate receptors were measured in XScore® (Wang *et al.*, 1998), and these values were established as baselines for comparison for each receptor subtype. The small molecules telmisartan and GW590735 were extracted from the ligand binding pockets of their cognate receptors and docked into that of their non-cognate counterparts. Conformational analysis was performed in each case and the affinity of the optimal conformation for its non-cognate ligand binding pocket was once more quantified in XScore® (Wang *et al.*, 1998). These optimal conformations were used in parallel processes. The first was a virtual screening exercise, using the molecular database ViCi (Lamzin, 2016), in which they were used as query molecules for the identification of spatially and electronically analogous structures capable of forging similar or enhanced interactions within the non-cognate ligand binding pockets. This process yielded molecular cohorts for each query molecule ranked in order of similarity to the query. The second was a fragment based *de novo* approach which was carried out using LigBuilder® v1.2 (Wang *et al.*, 2000). Here, molecular moieties from the telmisartan and GW590735 scaffolds considered critical to binding were identified and modelled in Sybyl® v1.2 (Tripos, 2010), in order to produce a number of seed structures capable of sustaining molecular growth at user directed sites designated as *H.spc* atoms subsequent to their being docked within the non-cognate ligand binding pockets. The docking process ensured that the created fragments were essentially superimposed onto the same *locus* within the non-cognate ligand binding pocket as that which they occupied when they formed part of the parent optimal conformer. Molecular growth was carried out using the Link and Grow Algorithms of LigBuilder® v1.2 (Wang *et al.*, 2000), and the resultant structures were, for each seed structure segregated into families of pharmacophoric similarity and ranked within each family according to ligand binding affinity. Physicochemical data including Molecular Weight and logP were also included, as was an indicator of synthetic feasibility. The molecular cohorts identified through both approaches were filtered for Lipinski Rule compliance. The molecules that survived filtering were then redocked into their original receptor ligand binding pocket, conformational analysis re-performed and the affinity of the optimal conformer measured for each using XScore® (Wang *et al.*, 1998). Comparison was made to the baseline and non-cognate receptor affinities initially

established, and the molecules exhibiting dual affinities exceeding baseline values were selected for further optimisation. This study has significant clinical potential. This derives not only from the notion of the proposed simultaneous dual agonism of 2 PPAR subtypes- PPARγ whose modulation is associated with a hypoglycaemic effect and PPARα whose modulation is associated with dyslipidaemia management, but also from the fact that the angiotensin receptor blocking scaffold of telmisartan would also have an anti-hypertensive effect, consequently creating a scenario in which the highly prevalent metabolic syndrome could be managed using one molecule. The facts that the angiotensin converting blocker and fibrate scaffolds of telmisartan and of GW590735 were largely conserved during the *de novo* design exercise, and that both of these have already been used clinically without unacceptable toxicity profiles is also advantageous.

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Poster communication 28

Construction of *De Novo* All-Carbon Quaternary Stereocentes in Unbiased Acyclic Systems

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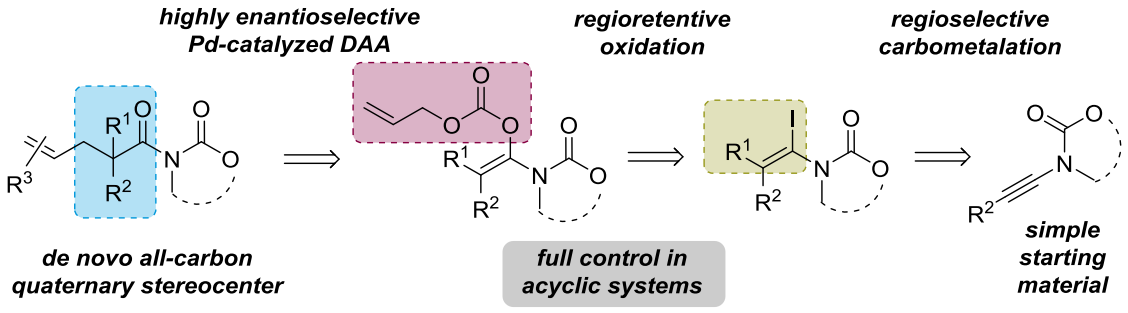
When four different carbon-based substituents are attached to a single carbon atom, they form a structure often referred to as an all-carbon quaternary stereocentre [1]. Such structures represent minimalistic molecular frameworks with enhanced (bio)chemical stability and embedded propensity to encode directionality in 3D space. Only a handful of methods exists to access these building blocks from simple starting materials in a stereospecific manner. In unbiased acyclic systems, typical chemical strategies will set distinct limits on the range of substituents that the method can be used for. Hence, more general routes to access the entire spectrum of differentially substituted enolates remain to be explored.

We developed an efficient protocol to prepare versatile building blocks containing *de novo* created all-carbon quaternary stereocentres in unbiased acyclic systems. This was achieved by (1) extending the scope of the original carbometalation–oxidation strategy [2] to access a wide set of fully-substituted, stereodefined amide enolates from ynecarbamates by using Grignard reagents and by (2) examining the interplay between the nature of palladium ligands and the pendant carbamate group of the amide enolate during catalytic decarboxylative allylic alkylation.



Scheme 1: Construction of *de novo* all-carbon quaternary stereocentres in acyclic systems.

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Poster communication 29

Electrochemical Sensing of Ligand Binding to Proteins Associated with Membranes

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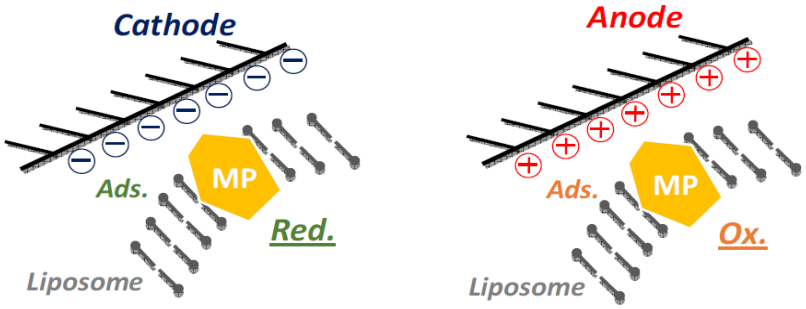
The development of new methods and strategies for the investigation of membrane proteins (MP) is limited by poor solubility of these proteins in an aqueous environment and hindered by a number of other problems linked to the instability of the proteins outside lipid bilayers. Therefore, current research focuses on an analysis of membrane proteins incorporated into model lipid membrane, most frequently liposomes.



In this contribution, we introduce a new electrochemical methodology for the analysis of transmembrane proteins reconstituted into a liposomal system. The proposed analytical approach is based on proteoliposomal sample adsorption on the surface of working electrodes followed by analysis of the anodic and cathodic signals of the reconstituted proteins (see Scheme). It works based on the fact that proteins are electroactive species, in contrast to the lipid components of the membranes under the given experimental conditions. [1] Electroanalytical experiments were performed with four model proteins associated with membranes, *i.e.* Na^+/K^+ATPase, protein ftt1103, mitochondrial uncoupling protein 1 and cytochrome *c*. Our results may contribute to the development of new electrochemical sensors and microarray systems applicable for monitoring of protein-ligand binding and evaluation of protein structure in general. [1,2]

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Poster communication 30

Monte Carlo method based QSAR modeling of dihydrofolate reductase inhibition by selected pyramidine derivatives

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In modern drug design process QSAR methods have an important role since they can make the early prediction of activity-related characteristics of drug candidates and therefore eliminate molecules with undesired properties. Conformation-independent methods have emerged as an attractive approaches for developing models since QSAR models since they don't require high computational resources and a long time period for computational experiments [1,2]. Monte Carlo optimization method applied for development QSAR models Monte Carlo method has been used as a computational tool for building QSAR models for the dihydrofolate reductase inhibition with 2,4-diamino-5-(substituted-benzyle)-pyramidine derivatives. Simplified molecular input line entry system (SMILES) together with hydrogen-suppressed graph (HSG), hydrogen-filled graph (HFG) and graph of atomic orbitals (GAO) were used to represent molecular structure and for descriptor calculation. One-variable models have been calculated for one data split into training, test set and validation. The impact of Morgan's extended connectivity index on built QSAR model and outliers was determined. Computational experiments indicated used methodology can satisfactorily predict desired endpoint. Structural indicators for the increase and the decrease of the studied activity are defined. Using defined structural alerts computer aided design of new 2,4-diamino-5-(substituted-benzyle)-pyramidine derivatives with desired activity is presented.

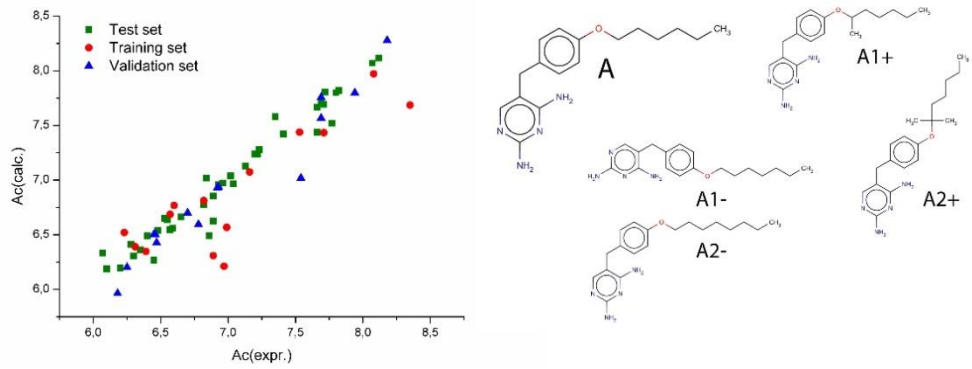

Figure 1: (Left) Graphical representation of best-developed QSAR model. (Right) The molecular design of perspective dihydrofolate reductase inhibitors using the developed QSAR model.

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Poster communication 31

New drug candidates targeting hydrogen sulfide

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Although hydrogen sulfide (H_2S) was considered as a toxic compound previously, now it is recognized as a gaseous intracellular signal transducer with important functions in neuromodulation, regulation of cardiovascular system and inflammation [1]. In the cardiovascular system H_2S causes angiogenesis, vasorelaxation, proliferation of vascular smooth muscle cells, protects myocardial cell from ischaemia and decreases reactive oxygen species. H_2S level has been found decreased in several diseases or conditions such as aging induced erectile dysfunction, diabetes, preeclampsia, alzheimer, epilepsy and down syndrome. H_2S-releasing derivatives of NSAID have shown promise in protection against gastric ulcer and in inflammatory bowel disease [2].

Interestingly resveratrol (RVT) have common mechanisms with H_2S such as KATP and SIRT activation, PDE inhibition and reactive oxygen species (ROS) inhibition and vasorelaxation in penile tissue and aorta [3, 4]. Thus we investigated if RVT (0.1 or 0.01 mM in CC and aorta, respectively) induces H_2S formation, relaxes the vascular tissues via H_2S or decreases ROS formation through induction of H_2S. We measured H_2S formation by methylene blue assay, concentration dependent relaxations to RVT and NaHS by DMT myograph in CD1 male mouse aorta and corpus cavernosum and measured ROS formation by luminol assay in oxidative stress induced by pyrogallol (0.1 mM). Cystathionine-gamma-lyase (CSE) inhibitor PAG or cystathionine-β-synthase (CBS) and CSE inhibitor aminooxyacetic acid (AOAA) (2 mM) or eNOS inhibitor L-NNA (0.1 mM, 30 min) was used to elucidate the role of H_2S pathways on the effects of RVT. One- or Two Way Anova was used as statistical test.

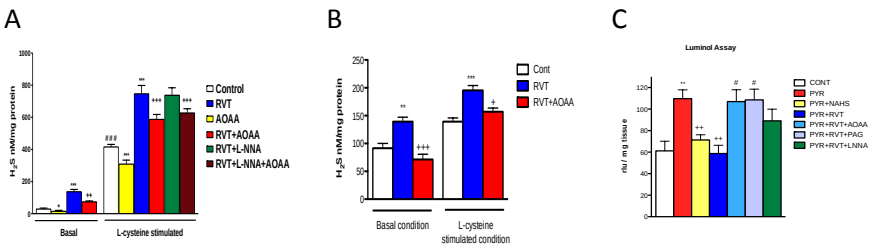

Figure 1: Figure 1: The effect of RVT on H_2S formation in A) MCC and B) mouse aorta and C) reactive oxygen species. RVT significantly increased H_2S level in basal and L-cysteine stimulated conditions and AOAA prevented this augmentation. *p<0.05,**p<0.01,***p<0.001 compared to control, +p<0.05, ++p<0.01,+++p<0.001 compared to RVT.

RVT stimulated both basal and L-cysteine-induced H_2S formation in both aorta and penile tissue. H_2S inhibitor AOAA or PAG inhibited the augmented H_2S formation and endogenous H_2S-dependent relaxation, suggesting the role of H_2S in vasorelaxant effect of RVT, (Figure 1). RVT relaxed MCC dose dependently (96.90± 4.26 vs 123.4±3.755) and this relaxation was inhibited by AOAA or AOAA+PAG (123.4±3.755 vs 96.85 ± 3.474 and 96.90 ±4.260, p<0.01, n=12, 8 and

5 respectively) but not by L-NNA[5]. RVT inhibited pyrogallol-induced radical generation in mouse aorta ($p<0.05$, $n=5$). AOAA significantly reversed the inhibitor effect of RVT on pyrogallol-induced ROS formation ($p<0.05$, $n=5$).

We concluded that RVT relaxes vascular tissues via H_2S formation through induction of H_2S via CBS/CSE pathway and may be beneficial in erectile dysunction or endothelial dysfunction. This study may be important to show the potential of H_2S-targeting drugs in cardiovascular diseases.

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Legend

PL = plenary lectures; O = oral communications; P = poster communications

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