Third Annual Meeting
CA15135

BOOK OF THE ABSTRACTS

COST ACTION CA15135

MuTaLig & Companies: the multi-targeting drug discovery with implications at industrial level

University of Malta - Campus Historical building
Saint Paul street, Valletta (Malta)

www.mutalig.eu

INTRODUCTION

The MuTaLig COST Action aims to link interactions among highly-qualified research teams working in the emergent Medicinal Chemistry branch, known as multi-target or poly-pharmacology. Started in 2016, our COST Action has passed over the "turning point". Therefore, to start properly the second part of our four year activities, the third annual meeting has been designed to treat a special issue related to the involvement of industrial partners in the MuTaLig COST Action. Experts from known EU companies will present their activities in plenary talks, depict the impact of the multi-target drug discovery issue and contribute in the development of this field. All MuTaLig community will be involved to push the scientific activities toward the application, possibly at industrial level. Taking into account the special issue "Multi-Target Drug Discovery: an opportunity for novel and repurposed bioactive compounds" currently running on the prestigious European Journal of Medicinal Chemistry, the meeting will host also a plenary lecture of the associate editor Prof. Paola Barraja. Moreover, 20 short oral and 48 poster communications from experts within the European countries adhering the MuTaLig COST Action will enrich the intensive program, that will be closed with round table about the central topic of the meeting. Thanks to the availability of Dr. Claire Shoemake and Prof. David Magri, our two MC Malta Members, to organize it, the conference is fixed in two days: October 18th - 19th 2018. The location is the historical University building, within the beautiful center of Valletta, recently nominated 2018 European Capital of Culture. The meeting participation is free (no fee), limited to 125 participants registered on the e-cost platform and properly invited from it. All additional updates will be posted at www.mutalig.ue.

As Chair of this COST Action, I want to express my gratitude especially to the local organizers and LOS, to their local team, 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. A special thank is also due to Dr. Antonio Lupia (Università "Magna Græcia" di Catanzaro, Italy) for the support in the organization of this abstract book.

I wish a fruitful and stimulating annual meeting to all participants!

Stefano Alcaro
Università "Magna Græcia" di Catanzaro (Italy)
Chair of CA15135 COST Action
alcaro@unicz.it

Local Organizing Committee

Claire Shoemake

David Magri

Acknowledgments

UNIVERSITY OF MALTA
L-Università ta' Malta

PROGRAM

Thursday October 18th 2018

8.30 Registration

9.00 **Introduction to the MuTaLig COST Action 3rd Annual meeting**
Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)
Lilian M. AZZOPARDI Head of Department of Pharmacy, University of Malta and President of the European Association of Faculties of Pharmacy

Session I: patents and anticancer agents
Moderator Nigel Richards (MC member for UK) - University of Cardiff, UK

9.15 PL1 **From Bench to Spin-of: How to reach results able to be patented**
Bruno BOTTA (MC substitute for Italy) - MoLiRom Srl spinoff, Roma (Italy)

9.45 OR1 **Discovery of a new small molecule with strong and selective *in vitro* and *in vivo* therapeutic activity in human lymphomas**
Eugenio GAUDIO (WG2 leader) - Oncology Research Institute, Bellinzona (Switzerland)

10.00 OR2 **Angiogenesis inhibitors: better if multi-targeted**
Ana QUESADA University of Malaga (Spain)

10.15 OR3 ***In vitro* evaluation of anticancer activity and *in silico* estimation of mechanisms of action of newly synthesized 9-aminoacridine derivatives**
Vladimir DOBRIČIĆ (MC member for Serbia) - University of Belgrade (Serbia)

10.30 OR4 **Inhibition of Glutamine Metabolism as a Multitarget Therapy against Pancreatic Ductal Carcinoma**
José M. PADRON (MC substitute for Spain) - Universidad de La Laguna, Tenerife (Spain)

10.45 *Coffee break*

Session II: proprietary products and neurodegeneration
Moderator Maria Laura BOLOGNESI (MC member for Italy) - "Alma Mater" Università di Bologna (Italy)

11.15 PL2 **Lead Discovery Siena: Development of Proprietary Products for Infective diseases and Oncology**
Annalaura BRAI Lead Discovery Siena Srl spinoff, Siena (Italy)

11.45 OR5 **Coumarin – One Core to Rule Them All**
Olli PENTIKÄINEN (MC member for Finland) - University of Turku (Finland)

12.00 OR6 **Structure-based Discovery of Dual Inhibitors of Disparate Drug Targets for Parkinson's Disease**
Jens CARLSSON (MC member for Sweden) Uppsala University, Sweden

12.15 OR7 **Discovery and Characterisation of Tacrine/Huprine-Tryptophan Heterodimers as Novel Multipotent Compounds Against Alzheimer's Disease**
Jan KORABECNY (MC member for Czech Republic) - University Hospital, Hradec Kralove (CZ)

12.30 OR8 **In the search for selective cholinesterase inhibitors against Alzheimer's disease**
Óscar LÓPEZ University of Sevilla (Spain)

12.45 OR9 **History in the Making: development of multi-target-directed ligands for Alzheimer's disease that modulate oxidative stress/mitochondria quality and the cholinergic system**
Sofia BENFEITO University of Porto (Portugal)

13.00 OR10 **ABAD (17β-HSD10) inhibitors with implications to neurodegenerative diseases and cancer treatment (from molecular design to in vivo data)**
Kamil MUSILEK (MC member for Czech Republic) - University of Hradec Kralove (CZ)

13.15 *Lunch*

Session III: against different (multi)targets
Moderator Maurizio BOTTA (Industrial coordinator of CA15135) - Università di Siena (Italy)

14.30 PL3 **Towards the discovery of new correctors of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) based on a repositioning study of photosensitizers**

Paola BARRAJA (Associate Editor of Eur. J. Med. Chem.) - Università di Palermo (Italy)

15.00 OR11 **Toward Dual Gyrase A/Gyrase B Inhibitors as Antibacterial Agents**
Danijel KIKELJ (WG1 leader) - University of Ljubljana (Slovenia)

15.15 OR12 **Increasing Functional Cellular Uptake of Small Molecules Using Deoxycholic Acid Conjugation**
Pavel STARKOV Tallinn University of Technology, Tallinn (Estonia)

15.30 OR13 **Rutin fatty acid esters as multi-target compounds**
Magdalena MAJEKOVA Slovak Academy of Sciences, Bratislava (Slovakia)

15.45 OR14 **Discovery of Novel Methyl Jasmonate Derivatives and Evaluation of Their Mechanisms in Cancer**
Mustafa GUZEL (MC substitute for Turkey) - Medipol University İnternational, Istanbul (Turkey)

16.00 OR15 **Trans-National Fraunhofer small molecule drug discovery projects**
Sheraz GUL (MC substitute for Germany) Fraunhofer Institute, Hamburg (Germany)

16.15 OR16 **Physicochemical clustering of pharmaceuticals in the WHO Anatomical Therapeutic Chemical Classification**
Claude FARRUGIA University of Malta, Msida (Malta)

16.30 *Coffee break and Poster session*

17.00 MC meeting (for MC members/substitutes only)

18.00 *Social activity (optional)* *www.um.edu.mt/events/mutalig2018/socialevent*

Friday October 19th 2018

Session IV : computational methods and multi-target drug design
Moderator Sharon BRYANT (WG3 leader) - Inte:Ligand GmbH, Vienna (Austria)

9.00 PL4 **Methods for protein ligand unbinding kinetics estimation**
Sergio DECHERCHI BIKI Technologies Srl spinoff, Genova (Italy)

9.30 OR17 **Machine-learning and ligand pharmacology for multi-target ligand design**
Alfonso GARCIA-SOSA (MC member EE, comunication manager CA15135) - University of Tartu (EE)

9.45 OR18 **Binding Estimation After Refinement (BEAR): a post-docking tool for drug design**
Giulio RASTELLI Università di Modena e Reggio Emilia (Italy)

10.00 PL5 **A Multi-level strategy for the generation of bioactive conformational ensembles**
Robert SOLIVA NOSTRUM Biodiscovery, Barcellona (Spain)

10.30 *Coffee break and Poster session*

Session V: GPCR ligands
Moderator Bart ROMAN (MC member for Belgium) – Ghent University (Belgium)

11.00 PL6 **Navigating Structural GPCR-Ligand Interaction Space for Computer-Aided Drug Design**
Chris DE GRAAF - HEPTARES therapeutics, Cambridge (UK)

11.30 OR19 **Case studies for succesful combination of cholinesterase inhibitors and GPCR ligands with procognitive *in vivo* activity**
Michael DECKER - Julius Maximilian University of Würzburg (Germany)

11.45 OR20 **In vitro, in silico and in vivo studies of D2AAK4 as a potential multi-target anti-psychotic**
Agnieszka Anna KACZOR Medical School of Lublin (Poland)

12.00 **Round table and best poster awarding ceremony**
Moderator Thierry LANGER (MC substitute for Austria) - University of Vienna (Austria)
Fernanda BORGES (CA15135 Vice-Chair) - University of Porto (Portugal)
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 (Industrial coordinator of CA15135) - Università di Siena (Italy)

Maria Laura BOLOGNESI (STSM coordinator of CA15135) - "Alma Mater" Università di Bologna, Bologna (Italy)
Alfonso GARCIA-SOSA (Comunication manager of CA15135) - University of Tartu (Estonia)

13.00 **Announcements for the next MuTaLig meetings and concluding remarks**
Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)
Sheraz GUL (MC substitute for Germany) Fraunhofer Institute, Hamburg (Germany)
Luc DEMANGE (MC member for France) Université Paris-Descartes, Paris (France)
Claire SHOEMAKE and David MAGRI (LOS and MT MC members) - University of Malta (MT)

Plenary lectures

Plenary Lecture 1

FROM BENCH TO SPIN-OF: HOW TO REACH RESULTS ABLE TO BE PATENTED

Bruno Botta

Dept of Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le Aldo Moro 5, Rome, Italy

bruno.botta@uniroma1.it

The mission of MoLiRom is focussed on the development of advanced projects in the synthesis, extraction and production of bioactive substances of natural origin and protein based products. MoLiRom offers a unique catalogue of natural products, protein specialties and chromatography products. Molirom is active in R&D projects and is currently participating to the H2020 Marie Curie Action INT "X-Probe". Molirom has developed a set of engineered ferritin proteins to be used as a scaffold for drug delivery and diagnostics. The priviledged scaffold is based on the thermostable ferritin from Archeglobus fulgidus, produced in high yield as a recombinant protein and easily assembled/disassembled as a function of divalent cation. The protein can host therapeutic or diagnostic molecules by means of "encapsulation" procedures while keeping with unique targeting properties towards the transferrin receptor. MoLiRom also possesses unique skills in the science of chemical separation and researchers are able to develop full downstreaming bioprocesses for protein purification and also small molecules extraction and separation.

Figure 1: From left to right: Dps and Ferritin proteins, Molirom's molecular biology lab, Scketch of a monolithic separation column.

Plenary Lecture 2

Lead Discovery Siena: Development of Proprietary Products for Infective diseases and Oncology

Annalaura Brai[a]

[a] *Lead Discovery Siena S.r.l., Castelnuovo Berardenga, Siena, Italy*

al.brai@leaddiscoverysiena.it

Lead Discovery Siena (LDS) is an innovative PMI focused on the drug research. LDS was founded as a spin-off of the University of Siena in 2012 by combining the scientific background and the entrepreneurship of its management to offer specific competences and solutions in drug discovery. LDS actively participates to national and international projects, with a special attention to local synergies at industrial and academic level within the Life Science area. In this context LDS is one of the two industrial members of the project UNAVIR, aimed at identifying new therapeutic treatments for rare viral infections and is the leading institution in PANVIR.NET a project focused on the preclinical development of novel panviral agents. LDS offers pharmaceutical services for Industry and Academia ranging from computational chemistry to custom synthesis, bioconjugation and ADME/PK analysis. LDS has a diversified pipeline of licensed and proprietary products[1-5] for oncology and infective diseases, including orphan and rare pathologies.

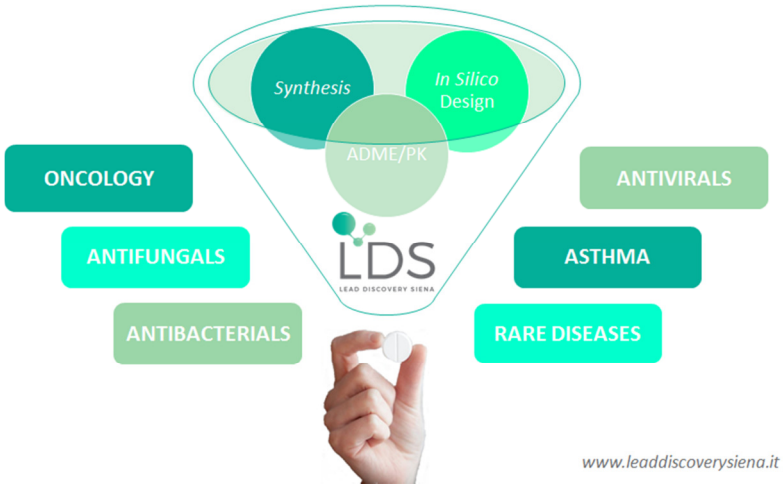

Figure 1: Services and project portfolio

[1] Botta M et al. (2009) European Patent Application No PCT/IB2009/051032, Linear And Cyclic Guanidine Derivatives, Method Of Preparation And Uses Thereof.

[2] Botta M et al. (2015) European Patent Application No PCT/EP2015/075148 Compounds and Uses Thereof.

[3] Botta M et al. (2016) European Patent Application No PCT/EP2016/052990 Human Helicase DDX3 Inhibitors As Therapeutic Agents.

[4] Botta M et al (2017) European Patent Application No PCT/EP2017/057010 Use Of DDX3 Inhibitors As Antiproliferative Agents.

[5] Botta M et al. (2014) European Patent Application No PCT/EP2014/062896 New Macrocyclic Amidinourea Derivatives, Methods Of Preparation And Uses Thereof As Chitinase Inhibitors

Plenary Lecture 3

Towards the discovery of new correctors of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) based on a repositioning study of photosensitizers

Paola Barraja

Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo (Italy).

paola.barraja@unipa.it

Several naturally occurring and synthetic structures are capable of photoactivation, few however allow this energy transfer to enable specific photochemical reactions. These agents, called photosensitizers (PSs) have found application in the treatment of several disorders, among which tumours. Their administration followed by light activation of a specific wavelength, induces a series of photobiological processes by energy transfer to molecular oxygen yielding $^1O_2$ and other reactive oxygen species (ROS) that cause rapid and irreversible cyto and vasculo toxicity to tumour tissues. In this contest, we have focused our attention for a long time in the identification of new PS agents, and new classes of small nitrogen heterocycles showed very promising photosensitizing properties with remarkable photoantiproliferative effect.[1] Linear and angular furocoumarins, studied for decades as PSs, have been proposed in the last years as multitarget agents in the possible treatment of Cystic fibrosis (CF), a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[2] Of the about 2000 known CF mutations, deletion of phenylalanine at position 508 (F508del) in the CFTR protein is the most common one.[3] At present two orally-bioavailable small molecule targeting mutant CFTR are commercially available for CF patients: the corrector VX-809 and the potentiator VX-770. We started our study aiming at finding new chemotypes to maximize the rescue of mutant CFTR for the correction of the basic defect and with a particular ability to synergize with first generation correctors. In fact, is generally accepted that treatment with a single corrector is not enough to achieve a clinically relevant rescue of F508del defect and that a combination of correctors having complementary mechanisms is desired. We used our collection of PSs, to generate a small library of nearly 200 compounds which were screened at the Telethon Institute of Genetics and Medicine (TIGEM) revealing one lead compound which showed an interesting ability to functionally rescue F508del-CFTR, particularly in combination with VX-809. Importantly, it was also active in primary bronchial epithelial cells producing a marked synergic effect on transepithelial chloride secretion. These results pave the way to develop highly effective F508del correctors, capable to synergize with class 1 correctors.

References

[1] see for example: Spanò, V.; Parrino, B.; Carbone, A.; Montalbano, A.; Salvador, A.; Brun, P.; Vedaldi, D.; Diana, P.; Cirrincione, G.; Barraja, P., *Eur. J. Med. Chem.* **2015**, *102*, 334–351; Spanò, V.; Giallombardo, D. ; Cilibrasi, V.; Parrino, B.; Carbone, A.; Montalbano, A.; Frasson, I.; Salvador, A.; Richter, S. N.; Doria, F.; Freccero, M.; Cascioferro, S.; Diana, P.; Cirrincione, G.; Barraja, P. *Eur. J. Med. Chem.* **2017**, *128*, 300–318; Spanò, V.; Frasson, I.; Giallombardo, D.; Doria, F.; Parrino, B.; Carbone, A.; Montalbano, A.; Nadai, M.; Diana, P.; Cirrincione, G.; Freccero, M.; Richter, S. N.; Barraja, P. *Eur. J. Med. Chem.* **2016**, *123*, 447−461.

[2] Cabrini, G.; Casavola, V.; Gambari, R. PCT Int. Appl. WO2012/171954.

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Plenary Lecture 4

Methods for protein ligand unbinding kinetics estimation

Sergio Decherchi

Istituto Italiano di Tecnologia, Via Morego, 30 16163 Genova, Italy

sergio.decherchi@iit.it

Drugs residence time has been reported to be a powerful indicator of the in vivo efficacy in clinical trials even more than drug binding affinity. For this reason, it would be convenient to have a computational tool able to predict the drug binding residence time. We devised a first protocol based on scaled molecular dynamics able to rank ligands based on their residence time. The protocol works well in practice but it does not give any mechanistic insights into the unbinding process. For this reason, we developed a second protocol that together with the ability to rank compounds based on the residence time is able to give mechanistic insights into the unbinding process. This method is rooted on the Adiabatic Bias Molecular Dynamics. We verified that the method works similarly to scaled MD but with additional advantages. We also prospectively applied the method to GSK-3beta where we collected experimental SPR data and new crystal structures.

Plenary Lecture 5

A Multi-level strategy for the generation of bioactive conformational ensembles

Sanja Zivanovic[1], Adam Hospital[1], Robert Soliva[2], Francesco Colizzi[1], Genis Bayarri[1], Modesto Orozco[1]

1 Institut de Recerca Biomedica, Barcelona, Spain

2 Nostrum Biodiscovery, Barcelona, Spain

Flexible molecules such as drugs and leads populate an ensemble of energetically accessible conformations amongst which the bioactive conformation, the one adopted in the protein-bound state, is found. There are many techniques to generate conformational ensembles for flexible ligands. However, many of them do not reliably select only the conformers that are most likely to bind to protein targets. We have developed a multi-level automatic pipeline to generate meaningful conformational ensembles of flexible ligands in solution by combining a classical force field-based enhanced-sampling technique (Hamiltonian replica exchange MD) with subsequent higher-level QM calculations. We have tested this methodology on a wide dataset of 100+ complexes of drug-like molecules. We could confirm the methodology is able to generate, in 70 % of the cases, conformations within 0.7 A Rmsd (internal coordinates) with respect to the bioactive conformation as found in the X-ray. Importantly, this is achieved in rather small conformational ensembles. Additionally, we find that in 71% of cases, the relative stability of the bioactive conformation is within 2 kcal/mol over the lowest energy conformer, at the QM level of theory employed. Amongst the molecules tested, we studied ligands found in the PDB co-crystallized with two different proteins, i.e., ligands with two bioactive conformations. We will discuss the data in the light of a multi-targeted strategy in small molecule drug discovery.

Plenary Lecture 6

Navigating Structural GPCR-Ligand Interaction Space for Computer-Aided Drug Design

Giovanni Bottegoni, Francesca DeFlorian, Robert T Smith, Juan Carlos Mobarec, Conor CG Scully, Jonathan S Mason, Miles Congreve, Benjamin G Tehan, Chris de Graaf

Heptares Therapeutics, The Steinmetz Building, Granta Park, Great Abington, Cambridge, CB21 6DG

Chris.Degraaf@heptares.com; Giovanni.Bottegoni@heptares.com

Novel crystal structures of GPCR-ligand complexes solved at Heptares and elsewhere continue to reveal a diversity of potential ligand binding sites, such as new allosteric binding sites for Class A and Class B GPCRs. This presentation will show how the breakthroughs in GPCR structural biology can be complemented by computational and experimental studies for a more accurate description and prediction of molecular and structural determinants of ligand-receptor binding affinity, kinetics, potency, and selectivity. Integrated cheminformatics workflows will be described that combine structural, pharmacological, and chemical data to explore receptor-ligand interaction space and steer structure-based virtual ligand screening. Orthogonal physics-based (Molecular Dynamics, e.g. Free Energy Perturbation FEP+, WaterMap from Schrödinger) and empirical (e.g. GRID and WaterFLAP from Molecular Discovery) structure-based drug design methods will be presented to target lipophilic hotspots, water networks, and cryptic ligand binding pockets for a variety of GPCR subfamilies.

Short communications

Short communication 1

Discovery of a new small molecule with strong and selective in vitro and in vivo therapeutic activity in human lymphomas

Eugenio Gaudio [1], Filippo Spriano [1], Chiara Tarantelli [1], Matilde Guala [2], Eugenia Riveiro [3], Gaetanina Golino [4], Antonio Lupia [4], Giosuè Costa [4], Roberta Rocca [4], Luciano Cascione [1], Stefano Alcaro [4], Francesco Paduano [4], Francesco Trapasso [4], Emanuele Zucca [1,5], Anastasios Stathis [5], Natalina Pazzi [2], Franco Cavalli [5], Francesco Bertoni [1]

[1] Università della Svizzera italiana, Institute of Oncology Research, Bellinzona, Switzerland; [2] Chimete, Tortona, Italy; [3] Early Drug Development Group, Paris, France; [4] University "Magna Græcia" of Catanzaro, Catanzaro, Italy; [5] Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.

Eugenio.gaudio@ior.usi.ch

Introduction. In the last few years, three small molecules, the PI3K inhibitor Idelalisib, the BTK inhibitor Ibrutinib and the BCL2 inhbitor Venetoclax have been approved by the FDA and have been flanked to Rituximab and to the traditional chemotherapy (e.g. CHOP) for the treatment of lymphoma's patients. Despite the improvements, still too many patients die for their diseases and more medicine are needed. We designed, tested and patented a new small molecule denominated EG-011 that demonstrated strong anti-cancer activity both *in vitro* and *in vivo* models of human lymphomas.

Methods. Lymphoma cell lines, including both B- and T-cells lymphomas, and solid tumor cell lines were exposed to a large range of concentrations of EG-011 as single agent for 72h, followed by MTT proliferation assay and IC50 calculation. Apoptosis assay was performed in primary cells collected from two healthy donors by measuring the annexin V by FACS. Xenografts were established s.c. into the left flanks of female NOD-SCID mice; treatment with EG-011 (200 mg/kg, i.p. 5 days per week) started with already established tumors.

Combinations were assessed in five cell lines (OCI-LY-1, OCI-LY-8, REC1, MINO, TMD8), exposed for 72h to increasing doses of EG-011 alone and with increasing doses of FDA approved drugs (rituximab, ibrutinib, venetoclax, lenalidomide and bendamustine). Synergy was assessed with Chou-Talalay combination index (CI): synergism (<0.9), additive (0.9-1.1), antagonism/no benefit (> 1.1).

Results. EG-011 presented a median IC50 of 2.25 µM across 62 lymphoma cell lines (95% C.I. 1-5µM). A higher activity was observed in a group of 21 cell lines that had a median IC50 of 250 nM. Among these there were 11 of the germinal center B cell (GCB) diffuse large B cell lymphomas (DLBCL) subtype (sensitive n=11/21, resistant n=9/41, $P < 0.05$), 4 belonging to mantle cell lymphoma (MCL) (sensitive n=4/21, resistant n=6/41, P n.s.), 3 from marginal zone lymphoma (MZL) (sensitive n=3/21, resistant n=2/41, P n.s.). EG-011 did not show any anti-proliferative activity in a panel of 23 solid tumor cell lines (IC50s > 10 µM).

A dose-depended increase of the cells in the sub-G0 phase (20-55%) was observed in OCI-LY-19 and REC1 cell lines after EG-011 exposure (at 500 nM and 2 µM) for 72h. Conversely, any cytotoxic effect in PBMCs from two healthy donors was observed after treatment at 1 and 10 µM for 24h and 48h.

In an in vivo xenograft experiment with the MCL REC-1 cell line, EG-011 delayed tumor growth (Day 6, Day 7, Day 9, $P < 0.05$) and tumor weight. At the end of the experiment, EG-011-treated tumors were 2.2-fold smaller than controls ($P < 0.001$).

When evaluated in combinations, EG-011 was synergistic with the anti-CD20 antibody rituximab in 5/5 cell lines. The combination with the Bcl2 inhibitor venetoclax was synergistic in 4/4 cell lines. The addition of the BTK inhibitor ibrutinib was synergistic in 2/2 ibrutinib sensitive cell lines, while the combination with the immunomodulant lenalidomide was beneficial in 3/3 lenalidomide sensitive cell lines (synergism in two and additivity in one). EG-011 plus bendamustine were synergistic in 5/5 cell lines.

Conclusion. The selective anti-lymphoma activity, in both *in vitro* and *in vivo* models, and the observed *in vitro* synergisms with FDA approved targeted agents make EG-011 a novel intriguing new drug candidate deserving further preclinical studies.

Short communication 2

ANGIOGENESIS INHIBITORS: BETTER IF MULTI-TARGETED

Ana R Quesada ,[a,b] Beatriz Martínez-Poveda,[a] Paloma Carrillo,[a] M. Carmen Ocaña-Farfán,[a] José Antonio Torres-Vargas,[a] Miguel Ángel Medina[a,b]

[a] *University of Malaga, Andalucía Tech, Department of Molecular biology and Biochemistry, Faculty of Sciences, Campus de Teatinos sn, Málaga, Spain*

[b] *U741 (CB06/07/0046) CIBER de Enfermedades Raras, Málaga, ES*

quesada@uma.es

Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is a main mechanism of vascularization during the embryonic development, growth, regeneration, wound healing and some physiological processes such as formation of the corpus luteum and endometrium. Abnormal angiogenesis is also involved in a number of pathological processes such as tumor growth, metastasis, diabetic retinopathy, age-related macular degeneration, psoriasis or arthritis, among others. For this reason angiogenesis inhibition has attracted broad attention in the field of pharmacological research. Clinical data indicate that angiogenesis inhibitors appear to be most effective when used in combination with other antiangiogenic or traditional anticancer therapies. The use of multi-targeted approaches to reach an effective inhibition of tumor angiogenesis arises as an attractive concept in antiangiogenesis[1]. Our group has a long trajectory in the search and characterization of new modulators of angiogenesis, in collaboration with research groups from Universities or Pharma companies. During this time a number of experimental procedures, including in vitro assays that resemble different steps of the angiogenic process, and some assays to test the in vivo angiogenesis inhibitory activity of the compounds have been developed[2]. As a fruit of our work, a number of compounds with remarkable antiangiogenic activity have been identified, either from natural or from synthetic origin. The biological activities of some of these compounds exhibit interesting multi-targeting profiles, what makes them interesting drug candidates for the treatment of angiogenesis-related diseases[3,4].

Our work is supported by grants PIE P12-CTS-1507 (Andalusian Government and FEDER) and BIO2014-56092-R (MINECO and FEDER). The “CIBER de Enfermedades Raras” is an initiative from the ISCIII (Spain).

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Short communication 3

In vitro evaluation of anticancer activity and in silico estimation of mechanisms of action of newly synthesized 9-aminoacridine derivatives

Vladimir Dobričić,[a] Jelena Pantić,[b] Jelena Grahovac,[c] Mara Aleksić,[b] Jasmina Brborić,[a] Siniša Radulović,[c] Olivera Čudina[a]

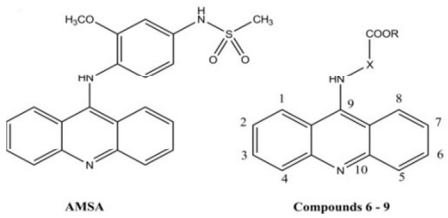
[a] *Dept of Pharmaceutical Chemistry, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia*

[b] *Dept of Physical Chemistry and Instrumental Methods, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia*

[c] *Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia*

vladimir@pharmacy.bg.ac.rs

A series of eleven 9-aminoacridine derivatives has been recently synthesized in our laboratory and their effects on cell viability examined and compared to amsacrine (AMSA)[1]. Compounds were tested in K562 and A549 cancer cell lines and in normal diploid cell line MRC5, and the viability was measured in tetrazolium based MTT assay[2]. The most potent compounds (**6-9**) had IC_{50} values similar or lower than AMSA (Figure 1). More importantly, toxicity of compounds **6**-**9** tested towards unstimulated normal human leucocytes was significantly lower in comparison to AMSA. Flow cytometry analysis of the cell cycle distribution of compounds **7** and **9** showed block in G2/M phase, whereas **6** and **8** did not induce significant effects.



6 (X = -CH2-CH2-CH2-, R = -CH2-CH3)
7 (X = -CH2-CH2-CH2-, R = -CH2-CH2-CH3)
8 (X = -CH2-CH2-, R = -CH2-CH3)
9 (X = -CH2-CH2-, R = -CH2-CH2-CH3)

Compound	K562	A549	MRC5
		IC50 ± SEM (μM)	
AMSA	13.8 ± 8.0	22.2 ± 2.8	15.4 ± 2.6
6	11.2 ± 0.4	9.5 ± 0.9	15.8 ± 3.2
7	21.8 ± 5.4	19.3 ± 4.0	16.9 ± 1.7
8	19.2 ±2.4	6.15 ± 0.6	12.1 ± 1.6
9	16.4 ±2.5	6.3 ± 0.2	11.6 ±1.3

Figure 1: Chemical structures of tested compounds and their IC_{50} values

In order to estimate mechanism of action of **7** and **9**, molecular docking studies were performed on several targets related to G2/M block: DNA - topoisomerase complex, cell division cycle protein 2 – cyclin B1 complex, checkpoint kinase 1 (Chk1), Wee1 kinase and α-tubulin-β-tubulin complex. Crystallographic structures of these targets in complex with inhibitors were taken from Protein Data Bank and molecular docking studies were performed in AutoDock Vina program. Both **7** and **9** form key binding interactions with DNA – topoisomerase complex and Chk1 and have similar binding energies to co-crystallized inhibitors, which indicates these compounds might act as multitarget inhibitors of cell-division cycle.

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Short communication 4

Inhibition of glutamine metabolism as a multitarget therapy against pancreatic ductal adenocarcinoma

José M. Padrón,[a] Miguel X. Fernandes, Alexis R. Galán

[a] *BioLab, IUBO AG, CIBICAN, Universidad de La Laguna, Astrofísico Fco. Sánchez 2, 38206 La Laguna, Spain*

jmpadron@ull.es

Pancreatic ductal adenocarcinoma (PDAC) cells have adapted to survive and proliferate in microenvironments under attack of the cells of the immune system, the deprivation of nutrients and oxygen, via mechanisms triggered by oncogenic KRAS. PDAC cells metabolism changes in response to O_2 and nutrient deprived environment. One of the most significant metabolic changes occurs in the glutamine pathway (Figure 1).[1] In normal cells, glutamine would enter the TCA cycle. But, in PDAC cells, glutamine is diverted to another route to generate NADPH, maintain cells redox balance and ensure proliferation. Enzymes of this glutamine pathway, reprogrammed by oncogenic KRAS, are GLS, GOT2, GOT1, MDH1 and ME1. This pathway is not used extensively by non-tumor cells and it was found that, in PDAC patients, high ratios of GOT1/GLUD1 or GOT2/GLUD1 were associated with worse survival. Our general objective is to demonstrate, in a preclinical setting, that the multitarget inhibition of GOT1, GOT2, MDH1 and ME1 with small molecules (SM) blocks PDAC growth. This approach represents the first time that multitarget inhibitors are being used to treat PDAC.

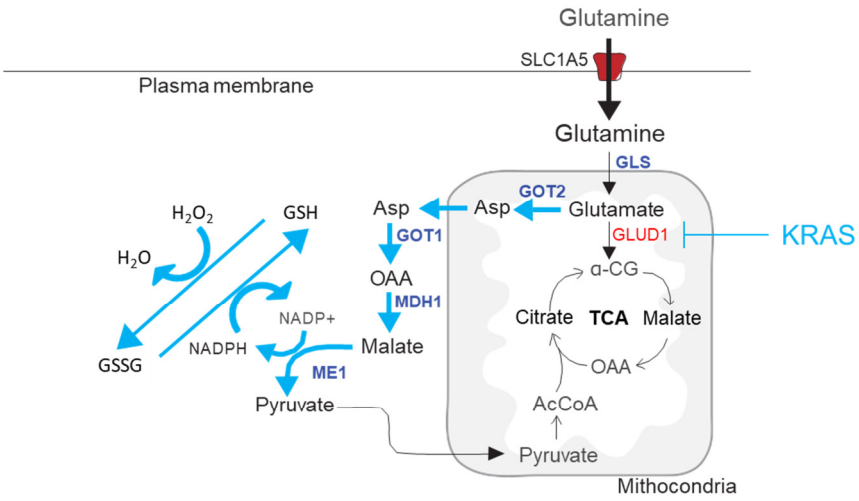

Figure 1: Glutamine pathway in KRAS mutated cells.

Multitarget SMs that inhibits GOT1, GOT2, MDH1 and ME1 own a high transfer potential. These SMs represent ideal candidates to obtain the designation of Orphan Drug from the European Medicines Agency to treat PDAC. Thus, our project is executed in close collaboration with the SME Orfan Biotech S. L.

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Short communication 5

COUMARIN – ONE CORE TO RULE THEM ALL

Olli Pentikäinen,[a,b] Sanna Niinivehmas,[a] Sakari Lätti,[a] Pekka Postila,[b] Sanna Rauhamäki,[b] Mira Ahinko,[b] Mira Pasanen,[b] Elina Multamäki,[b] Manivannan Elangovan,[b] Risto Juvonen,[c] Hannu Raunio,[c] Niina Nyberg,[c] Pasi Huuskonen,[c] Markku Pasanen,[c] Pasi Koskimies,[d] Johanna Troberg,[e] Moshe Finell,[e] Sami Kortet,[b,f] Emmi Schildt,[b,f] Juhani Huuskonen,[f]

[a] *Institute of Biomedicine, University of Turku, Turku, Finland*

[b] *Dept of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland*

[c] *School of Pharmacy, University of Eastern Finland, Kuopio, Finland*

[d] *Forendo Pharma, Turku, Finland*

[e] *Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Helsinki, Finland*

[f] *Dept of Chemistry, University of Jyväskylä, Jyväskylä, Finland*

olli.pentikainen@utu.fi

Coumarin is a privileged scaffold in medicinal chemistry. A huge number of versatile coumarin derivatives have been designed, synthesized, and tested to address many pharmacological targets. Easy synthetic transformations from low-cost starting materials make coumarin an attractive scaffold. Our group has created a library of coumarins to multiple target proteins, e.g. Estrogen receptor (ER), 17-beta-hydroxysteroid dehydrogenase 1 (HSD1), aromatase, monoamine-oxidase B (MAO-B), and UDP-glucuronosyltransferase enzymes (UGTs).

UGTs glucuronidate many pharmaceuticals in drug metabolism. We synthesized six new UGT1A10 substrates. All new derivatives are highly fluorescent, and their fluorescence decreases upon enzymatic glucuronidation. [1] **HSD1** and **aromatase** have crucial role in the E2 biosynthesis, and thus influences e.g. in breast cancer and endometriosis. Nine potent HSD1 and one potent aromatase inhibitors were identified.[2] Furthermore, coumarin core can be tailored to target **ER**. [3] **MAO-B** catalyzes deamination of monoamines like dopamine, and its inhibitors could alleviate the symptoms of neuropathologies like depression. The IC50 value of the most potent coumarin derivative was 56 nM. [4]

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Short communication 6

Structure-based Discovery of Dual Inhibitors of Disparate Drug Targets for Parkinson's Disease

Mariama Jaiteh[a], Alexey Zeifman[a], Marcus Saarinen[b], Per Svenningsson[b], Jose Brea[c], Maria Isabel Loza[c], Jens Carlsson[a]

[a] Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, BMC Box 596, SE-751 24 Uppsala, Sweden.
[b]Center of Molecular Medicine, Department of Physiology and Pharmacology, Karolinska Institute, SE-171 77 Stockholm, Sweden.
[c]USEF Screening Platform-BioFarma Research Group, Centre for Research in Molecular Medicine and Chronic Diseases, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain.

Email: jens.carlsson.lab@gmail.com

Modulation of multiple biological targets with a single drug can lead to synergistic therapeutic effects and has been demonstrated to be essential for efficient treatment of CNS disorders. However, rational design of compounds that interact with several targets is very challenging. Here, we demonstrate that structure-based virtual screening can guide the discovery of multi-target ligands of unrelated proteins relevant for Parkinson's disease.[1] A library with 5.4 million molecules was docked to crystal structures of the A_{2A} adenosine receptor (A_{2A}AR) and monoamine oxidase B (MAO-B). Twenty-four compounds that were among the highest ranked for both binding sites were evaluated experimentally, resulting in the discovery of four dual-target ligands. The most potent compound was an A_{2A}AR antagonist with nanomolar affinity (K_i = 19 nM) and inhibited MAO-B with an IC_{50} of 100 nM. Optimization guided by the predicted binding modes led to the identification of a second potent dual-target scaffold. The two discovered scaffolds were shown to counteract 6-hydroxydopamine-induced neurotoxicity in dopaminergic neuronal-like SH-SY5Y cells. Structure-based screening can hence be used to identify ligands with specific polypharmacological profiles, providing new avenues for drug development against complex diseases.

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Short communication 7

Discovery and characterization of tacrine/huprine-tryptophan heterodimers as novel multipotent compounds against Alzheimer's disease

Jan Korabecny,[a] Katarina Spilovska,[a,b] Manuela Bartolini,[c] Barbara Monti,[c] Doriano Lamba[d], Rosanna Caliandro,[d] Alessandro Pesaresi,[d] Vendula Hepnarova,[a] Daniel Jun,[a] Martina Hrabinova,[a] Rafael Dolezal,[a,b] Jana Zdarova Karasova,[a] Ondrej Soukup,[a] Eva Mezeiova,[a] Eugenie Nepovimova,[b] Maria Laura Bolognesi,[c] Kamil Kuca,[a,b]

[a] *Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 81, 500 05 Hradec Kralove, Czech Republic*

[b] *Department of Chemistry, University of Hradec Kralove, Rokytanskeho 62, 500 03 Hradec Kralove, Czech Republic*

[c] *Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy*

[d] *Istituto di Crystallografia, Consiglio Nazionale delle Ricerche, Area Science Park-Basovizza, S.S. 14-Km 163.5, I-34149 Trieste, Italy*

korabecny.jan1@gmail.com

Combination of tacrine/huprine, connected through a different linker tether length, with tryptophan led to the generation of a novel, highly-potent family of multi-target directed ligands targeting key molecular mechanisms of Alzheimer's disease.[1] Based on in vitro biological profile, the 6-chloro-tacrine-$(CH2)_6$-*L*-tryptophan heterodimer *S*-K1035 was found to be the most potent inhibitor of human acetylcholinesterase (*h*AChE) and human butyrylcholinesterase (*h*BChE) within the series, with nanomolar IC_{50} values (6.31 and 9.07 nM, respectively). Moreover, *S*-K1035 showed good ability to inhibit $A\beta_{42}$ self-aggregation and *h*AChE-induced $A\beta_{40}$ aggregation. The X-ray crystallographic analysis of *Tc*AChE in complex with *S*-K1035 highlighted the utility of the hybridization approach used in the structure based drug design. *S*-K1035 also exerted moderate inhibition against neuronal nitric oxide synthase (nNOS). *In vivo* studies displayed low toxicity profile compared to parent tacrine. *S*-K1035 also significantly ameliorated performances of scopolamine-treated animals.

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Short communication 8

In the search for selective cholinesterase inhibitors against Alzheimer's disease

Óscar López,[a] Jesús M. Roldán-Peña,[a] Daniel Alejandre-Ramos,[a] Inés Maya,[a] Antonio Franconetti,[b] Irene Lagunes,[c] José M. Padrón,[c] Luis E. Peña-Altamira,[d] Sabrina Petralla,[d] Eleonora Poeta,[d] Marina Naldi,[d] Manuela Bartolini,[d] Barbara Monti,[d] Maria L. Bolognesi,[d] José G. Fernández-Bolaños[a]

[a] *Depto. de Química Orgánica, Facultad de Química, Univ. de Sevilla Apartado 1203, E-41071, Seville, Spain*

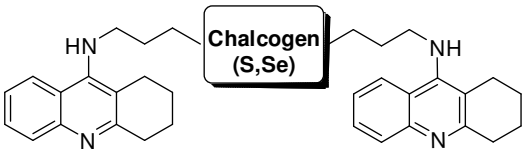
[b] *Dep. de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain*

[c] *BioLab, Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), CIBICAN, Univ. de La Laguna, C/ Astrofísico Francisco Sánchez 2, E-38206 La Laguna, Spain*

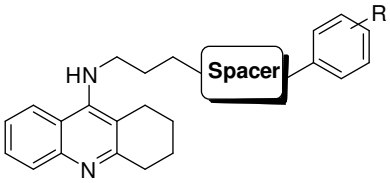
[d] *Dep. of Pharmacy and Biotechnology, Alma Mater Studiorum Univ. of Bologna, via Belmeloro 6, 40126 Bologna, Italy*

osc-lopez@us.es

Alzheimer's disease is currently considered the most relevant neurodegenerative disorder.[1] Moreover, its inherent complexity, makes it to be the prototype of a multifactorial disease, as even some of etiologies are not even fully understood.[2] The main therapies devoted to ameliorate the symptoms of the initial stages of Alzheimer's disease are based on the inhibition of acetylcholinesterase (AChE) and butirylcholinesterase (BuChE), two pivotal enzymes that control the hydrolysis of the neurotransmitter acetylcholine in the synapsis, the level of this compound being particularly low in Alzheimer's disease.[3] In this context, we have developed two different families of selective cholinesterase inhibitors derived from tacrine: chalcogen-containing homo- and heterodimers[4] and tacrine-aromatic residues hybrids (Figure 1). Anti-Alheimer's profile was analyzed for such compounds



Selective AChE inhibitors
(low nanomolar range)



Selective BuChE inhibitors
(subnanomolar range)

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Short communication 9

History in the Making: development of multi-target-directed ligands for Alzheimer's disease that modulate oxidative stress/mitochondria quality and the cholinergic system

S. Benfeito[a], F. Cagide[a], C. Oliveira[a], C. Fernandes[a], J. Teixeira[a,b], R. Amorim[a,b], J. Garrido[a,c], C. Martins[d,e], B. Sarmento[d,e,f], F. Remião[g], E. Uriarte[h], P.J. Oliveira[b], F. Borges[a]

[a]*CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal*

[b]*CNC, Center for Neuroscience and Cell Biology, UC-Biotech, University of Coimbra, Cantanhede, Portugal*

[c]*Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic of Porto, Porto, Portugal*

[d]*i3S, Institute of Investigation and Innovation in Health, University of Porto, Porto, Portugal*

[e]*INEB, Institute of Biomedical Engineer, Nanomedicines & Translational Drug Delivery Group, University of Porto, Porto, Portugal*

[f]*CESPU, Institute of Investigation and Advanced Formation in Sciences and Heath Technologies, Gandra, Portugal*

[g]*UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;*

[h]*Applied Chemical Science Institute, Autonomous University of Chile, Santiago de Chile, Chile*

sofia_benfeito@hotmail.com

Neurodegenerative diseases are progressive neurological disorders associated with central nervous system (CNS) dysfunction. In Alzheimer's disease (AD), the most prevalent disorder, etiology is multifactorial and neuronal death is the result of a complex network of cross-talking pathological stimuli over an extended period of time. One mechanism thought to contribute to AD is related with the overproduction of intracellular reactive oxygen species (ROS) by the mitochondria, which causes oxidative damage and impairs mitochondrial function. Moreover, the impaired of cholinergic transmission caused by acetylcholine depletion, and consequent synaptic changes in brain specific areas, has also a key role on the process. Despite major advances in understanding the factors that trigger AD to date no breakthrough treatment has yet been discovered. The current single-target treatments are only palliative and fail to modify disease progression. Accordingly, the development of multitarget-directed drugs (MTDDs), targeting cholinesterase's and mitochondria is attracting progressively more attention, as they can tackle intricate network effects. In that way, the use of mitochondriotropic antioxidants endowed with cholinesterase inhibitory activity seem to be an effective strategy.

In this work, dietary phenolic acids, such as naturally occurring hydroxycinnamic and hydroxybenzoic acids (HCA and HBA, respectively), were structurally modified to increase their mitochondria druggability, maintaining inherent antioxidant properties. Moreover, the assessment of *in vitro* antioxidant activity, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities, *in vitro* blood-brain barrier permeability and neuroprotective properties along with the evaluation of cytotoxic profile on differentiated human neuronal (SH-SY5Y) cell line has been performed. The results obtained so far will be presented in this communication.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145-FEDER-006980, PTDC/DTP-FTO/2433/2014 and NORTE-01-0145-FEDER-000028). S. Benfeito, F. Cagide, C. Oliveira, C. Fernandes, J. Teixeira and R. Amorim grants were also supported by FCT and FEDER/COMPETE and NORTE 2020 funds.

Short communication 10

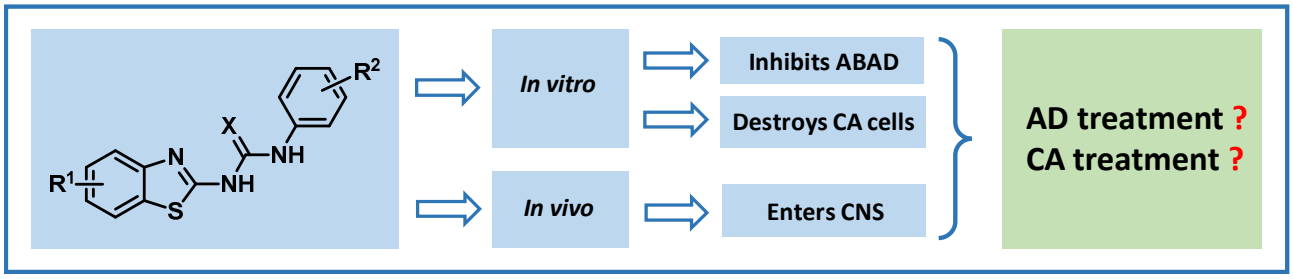
ABAD (17β-HSD10) inhibitors with implications to neurodegenerative diseases and cancer treatment (from molecular design to *in vivo* data)

Kamil Musilek,[a-b] Ondrej Benek,[a-b] Lukas Hroch,[b] Lucie Vaskova,[a-b] Monika Schmidt,[a-b] Tomas Kucera,[c] Katarina Nikolic,[d] Jan Misik,[b-c] Adam Skarka,[a] Laura Aitken,[e] Kamil Kuca,[a-b] Frank Gunn-Moore[e]

[a] *University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic*
[b] *University Hospital, Biomedical Research Centre, Hradec Kralove, Czech Republic*
[c] *University of Defence, Faculty of Military Health Sciences, Department of Toxicology and Military Pharmacy, Czech Republic*
[d] *University of Belgrade, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia*
[e] *University of St. Andrews, School of Biology, St. Andrews, KY16 9TF Fife, UK*

kamil.musilek@uhk.cz

Mitochondrial amyloid-binding alcohol dehydrogenase (ABAD), also known as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10), has been recognized to interact with amyloid-beta peptide (Aβ), which may lead to pathological changes in mitochondria and cell metabolism of Alzheimer's disease or cancer cells.[1] Aβ-ABAD interaction and altered enzyme function was shown to cause mitochondrial distress and consequent cytotoxic effect and thus it is a feasible target for drug development.



We have designed, prepared and evaluated non-competitive ABAD (17β-HSD10) inhibitors of benzothiazolyl structural scaffold that were found effective in low micro molar range.[2-3] Some novel compounds were found bioavailable after p.o. administration in mice/rats and brain penetrable. Other series of ABAD inhibitors was found relatively cytotoxic for selected cancer cell lines or inhibiting selected interleukins.

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Short communication 11

Toward Dual Gyrase A/Gyrase B Inhibitors as Antibacterial Agents

B. Fois[1,2*], Ž. Skok[2], M. Durcik, L. Peterlin Mašič, T. Tomašič[2], Á. Nyerges[3], J. Ilaš[2] and D. Kikelj[2]

[a] Department of Life and Environmental Sciences, University of Cagliari, via Ospedale 72, Cagliari 09124, Italy

[b] Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

[c] Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

danijel.kikelj@ffa.uni-lj.si

Bacterial DNA gyrase, an ATP-fueled heterotetrameric protein, composed of two A subunits (GyrA) and two B subunits (GyrB), is essential for cell viability because it introduces negative supercoils in DNA in front of the replication fork. The GyrA subunit is the target of fluoroquinolone antibiotics, while the GyrB ATP-binding site is a target of novobiocin and a number of recently reported GyrB inibitors. Noviobicin, discovered in the mid-1950s, was withdrawn from the market primarily due to its toxicity and due to the high resistance development and so far, no GyrB inhibitor has been introduced into the clinic. In previous works we reported the binding mode of several structural types of pyrrole-2-carboxamide derivatives e.g. 2-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-2-oxoacetic acid with *E. coli* GyrB.[2] Unfortunately, all these inhibitors did not show *in vitro* antibacterial activity, because of insufficient penetration and effluxing. It is assumed that dual targeting could reduce bacterial resistance because mutations at two different sites are less probable to occur than single mutation in GyrA and GyrB sites. These observations evoked our interest in dual targeting of gyrase A and gyrase B that could open new avenues for gyrase inhibition and fighting bacterial resistance.

Since gyrase A inhibitors (fluoroquinolones) and our pyrrole-2-carboxamide gyrase B inhibitors do not share common structural features, we decided to combine ciprofloxacin, through a methylene linker, with our recently reported benzothiazole-based gyrase B inhibitors, placing a pyrrole-2-carboxamide moiety at position 2 or 6 of the benzothiazole scaffold. Further, we hoped that ciprofloxacin would enable the molecules to penetrate into the bacteria and boost their antibacterial activity. All four prepared dual compounds displayed potent bioactivity against Gram negative *E.coli*. Moreover one compound showed good in vitro activity against Gram negative *Shigella flexneri* and *Klebsiella pneumoniae* and Gram-positive *S. aureus*. The lack of bioactivity change in presence of GyrB E136 mutant and the lack of bioactivity in the case of the mutated fluoroquinolone binding site (*E.coli* K-12 MG1655 GyrA S83L+D87N; ParC S80I+E84G) indicates that the primary binding site of these dual inhibitors in bacterial cell is GyrA and/or topoisomerase IV ParC. We have demonstrated that hybrids obtained by merging a gyrase B inhibitor with ciprofloxacin enter Gram negative and Gram positive bacteria and are not intensively effluxed. Further optimization of compounds is in progress.

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Short communication 12

Increasing Functional Cellular Uptake of Small Molecules Using Deoxycholic Acid Conjugation

Rohit Bhadoria, Kefeng Ping, Christer Lohk, Ivar Järving, Pavel Starkov*

Dept of Chemistry & Biotechnology, Tallinn University of Technology, Tallinn 12618, Estonia

pavel.starkov@ttu.ee

We show that conjugation of intracellularly active small molecules to deoxycholic acid (DCA) acts as a robust tool to increase cellular permeability of the linker-extended constructs without disrupting their cell-biological outcome. By examining induction of the phenotype of interest by using established non-fluorescent orthosteric and allosteric cytoskeletal probes as warheads, we show that these DCA-constructs act superiorly to alternative solutions by being less cell cytotoxic, having a larger dynamic range and reproducing the phenotype of interest across short and long timespans.

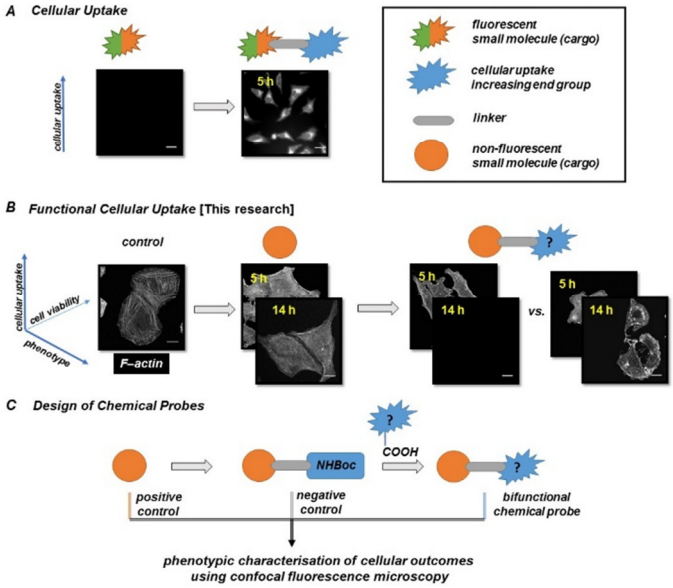

Figure 1: (A,B) Comparison of cellular uptake (by using fluorescent dye/cargo conjugates) vs. functional cellular uptake (observing changes in phenotype by using heterobivalent constructs and treatment with fluorescently-labelled small molecules/antibodies). **(C)** A unifying workflow for phenotypic characterization of differentially-modified constructs accessed by using protecting-group-free amide coupling, where a biofunctional chemical probe is supplemented by both positive and negative controls.

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Short communication 13

RUTIN FATTY ACID ESTERS AS MULTI-TARGET COMPOUNDS

Magdaléna Májeková,[a] Jana Viskupičová,[a] Yoel Rodríguez,[b] Ľubica Horáková [a]

[a] *Dept of Biochemical Pharmacology, Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, SAS, Dubravska Cesta 9, Bratislava, Slovakia*

[b] *Dept of Natural Sciences, Hostos Community College Of Cuny, 475 Grand Concourse, Bronx, 10451, New York, United States of America*

e-mail: magdalena.majekova@savba.sk

The acyl derivatives of rutin and other polyphenolic compounds, which are substituted with fatty acid chain length of 16–22, showed polypharmacologic effects targeting different proteins acting in chronic disease development and progression – trypsin, thrombin, urokinase and SERCA1. SERCA1, an isoform of sarco/endoplasmic reticulum Ca2+-ATPase (SERCA1 isoform), is one of the key proteins maintaining the cell calcium homeostasis and calcium signaling. Rutin esters as well as rutin itself, induced a significant loss of free sulfhydryl groups, protected the enzyme from protein carbonyl formation and prevented SERCA from tyrosine nitration (except R20:4 and R22:1) concerning the posttranslational modifications of SERCA1. While rutin stimulated SERCA1, rutin esters inhibited its activity. We focused our study also on detailed structural features of the interaction between rutin derivatives and SERCA1. Arachidonate derivative of rutin showed the highest polypharmacology potential among the compounds studied.

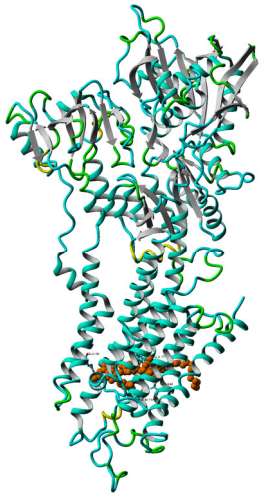

Figure 1: Rutin arachidonate in SERCA1

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Short communication 14

Discovery of Novel Methyl Jasmonate Derivatives and Evaluation of Their Mechanisms in Cancer

Mustafa Guzel [1,3], Bilgesu Onur Sucu[1] , Ozgecan Savlug[1] , Sukran Ozdatlı[1], Busra Emine Yazici[1]

[1] *İstanbul Medipol University, Regenerative and Restorative Medicine Research Center (REMER), Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810, Turkey*

[2] *Istanbul Medipol University, School of Medicine, Department of Medical Biology, Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810, Turkey*

[3] *Istanbul Medipol University, International School of Medicine, Department of Medical Pharmacology, Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810, Turkey*

mguzel@medipol.edu.tr

Cancer is one of the well-known illnesses leading to death. The Warburg effect describes the particular reliance of cancer cells on glycolysis for energy. Increased glycolysis and acid resistance have been postulated to be an essential part of carcinogenesis, conferring a significant growth advantage as well as promoting typical tumor progression [1]. Targeting accelerated glycolysis in cancer cells is a new promising modality for treatment of cancer. Inhibition of glycolysis can be done without significant side effects, and such treatment will be additive to most known cancer therapies. One way to inhibit the metabolism of cancer cells is to inhibit Hexokinase 2 (HK2) enzyme. HK2 has been studied in the field of cancer metabolism and hopeful results obtained. It has been also confirmed that HK2 enzyme is expressed 10-15 times more in cancer cells than normal cells. HK2 can bind to the mitochondrial VDAC (Voltage-dependent anion channel) [2] and VDAC is also overexpressed in cancer cells [3]. One of the anti-cancer strategy that targets energy metabolism of the cell is use of HK2 inhibitors to stop tumour growth.

Inhibition of HK2 enzyme will prevent cancer cells from nutrition and and it is expected that speeding of cancer cells will presumably stop tumor growth. Recent studies show that Methyl Jasmonate reveals promising results for treatment of cancer as a HK2 inhibitor. Cis-jasmone, Jasmonic acid and Methyl jasmonate are cyclopentanones that are fatty acid derivatives. Jasmonates are plant stress hormones which exhibit abnormal anti-cancer activity [4,5]. Jasmonates induced suppression of cell proliferation and death in a variety of cancer cell lines and cytotoxicity to cervical cancer cells with almost no effect on normal primary human keratinocytes [5]. It has been reported that Methyl Jasmonate binds specifically to mammalian hexokinase and disrupts its interaction with VDAC, and causes detachment of hexokinase from the mitochondria followed by cytochrome c release [6].. It is also reported that Jasmonic acid and methyl jasmonate are able to inhibit four isoforms of human aldo-keto reductase superfamily (AKR1C) [7].

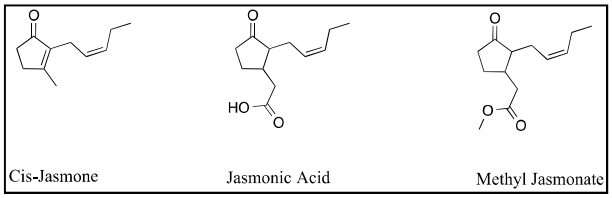

Figure 1: Cis-Jasmone, Jasmonic Acid, Methyl Jasmonate and Novel Methyl Jasmonate Analogues

In our research laboratory, we designed and synthesized handfull of novel methyl jasmonate analogs that has the multiple target potential for HK2 as well AKR1C inhibition and investigated their biological activity and anti-cancer effects on cancer cells from different origins. We will highlight the biological activity of those novel analogs as anti-cancer agents in our oral presentation.

Keywords Cancer metabolism, hexokinase inhibitors, methyl jasmonate, anti-cancer drugs

Note: This COST project (215S890) is funded by TUBITAK. We kindly appreciate for their support.

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Short communication 15

TRANS-NATIONAL FRAUNHOFER SMALL MOLECULE DRUG DISCOVERY PROJECTS

Sheraz Gul[a], Yulia Hering[a], Philip Gribbon[a], Jeanette Reinshagen[a], Francoise Halley[a].

[a]*Fraunhofer Institute for Molecular Biology & Applied Ecology (IME) – Screening Port (SP), Schnackenburgallee 114, D-22525, Hamburg, Germany.*

Sheraz.Gul@ime.fraunhofer.de

The Fraunhofer Institute for Molecular Biology & Applied Ecology – Screening Port (Fraunhofer-IME-SP) in Hamburg, Germany offers access to industry standard pre-clinical drug discovery know-how and complementary infrastructure. The overarching goal of the research activities at Fraunhofer-IME-SP are the development of diagnostic tools and therapies for various human diseases. Many of the trans-national networks involve assay development, High Throughput Screening (HTS), image analysis, data processing/mining, intellectual property, commercialisation strategies, compound characterisation and progression to the Lead and Clinical Candidate stages in drug discovery.

Figure 1: top left: the HTS system at the Fraunhofer for assay development and automated screening; **top middle:** automated HTS plate handling; **top right:** label-free HTS plate; **bottom left:** 3D cell-culture assay plate; **bottom middle:** automated HTS plate handling; **bottom right:** automated preparation of assay ready HTS plates.

Examples of trans-national projects include those that focus on cancer, neglected parasitic and cardiovascular disease. We will present work that has led to the development and implementation of a cell-based assay to discover agonists of the nuclear receptor REV-ERBα[1].

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Short communication 16

Physicochemical Clustering of Pharmaceuticals in the WHO Anatomical Therapeutic Chemical Classification

Claude Farrugia, Kevin Wood, Francesca Pace, Frederick Lia

Dept of Chemistry, University of Malta, Msida, Malta

claude.farrugia@um.edu.mt

The Anatomical Therapeutic Chemical (ATC) classification system managed by the World Health Organisation is considered the most widely used drug classification system.[1] Medicinal compounds are categorised into fourteen main classes, each representing the organ or system on which the medicinal compound acts. However, other classifications exist, based on different properties of the drug molecules, and with each system having its own advantages and limitations. The objective of this study was to propose a classification of the drugs in the ATC classification system, based on their physicochemical properties.

A total of 2530 drugs was included in the study. For each identified medicinal substance the physicochemical parameters of molecular density, total surface area, polar surface area, Log P, parachor, molecular weight and solubility at pH 7.4 were generated using computational methods and statistically assessed using several methods, including Multivariate Platform, Principal Component Analysis and K-Means Clustering. Four additional physicochemical parameters of each drug were subsequently computationally generated, namely atom count, hydrogen bond acceptor count, hydrogen bond donor count, and refractivity, and the statistical analyses repeated.

Results obtained with both the Multivariate Platform and the Principal Component Analysis exhibited a lack of correlation between solubility at pH 7.4 and the other physicochemical properties investigated. Elimination of solubility as a physicochemical parameter permitted K-means clustering of the medicinal compounds to form a classification of the drugs based on their remaining physicochemical parameters, called the PC1 classification. The elimination of various medicinal substances having negligible solubility and very high solubility values resulted in an increase in correlation between solubility at pH 7.4 and other physicochemical parameters. Hence, a second new classification system based on all the physicochemical parameters including solubility at pH 7.4, called the PC2 classification, was permitted via K-Means clustering. A third, PC3, classification was also developed using the additional physicochemical parameters. The hypothesis that a relationship exists between the above-mentioned classification systems was analysed by comparing the PC classifications with the ATC classification using three different statistical tools, namely, Supervised Linear-Canonical Discriminant Analysis, Linear Regression and Artificial Neural Network Analysis. It was concluded that all the new classifications were statistically different from the ATC classification, but may be useful as a tool to identify the potential physicochemical classification of lead compounds.

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Short communication 17

Machine-learning and ligand pharmacology for multi-target ligand design

Alfonso T. García-Sosa[a]

[a] *Institute of Chemistry, University of Tartu, Ravila 14a, Tartu, 50411, Estonia*

alfonsog@ut.ee

In multi-target drug design, it is important to distinguish actives and in-actives from among a variety of targets and anti-targets. Machine-learning techniques can use a variety of mathematical relationships and adjust them to observed and predicted classes or activities. Drugs and non-drugs were separated using decision tree, random forests (RF), support vector machine (SVM), artificial neural network (ANN), *k*-nearest neighbors (kNN), and logistic regression models, as well as an ensemble learning method.[1] In addition, drugs belonging to cardiovascular, anti-neoplastic, as well as nervous system disease classes were able to be separated.[1,2] Ligand features can help thus design drugs which can have activity in a variety of targets either in the same organ, or in different organs/diseases.[2,3] Therefore, ligand structure can encode multi-target activity, in a good way, such as affecting several targets related to the same disease, or in a way to be avoided, such as those with unspecific, assay-interfering behaviour. Methods to calculate multi-target behaviour by ligand structure will be presented.

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Short communication 18

BINDING ESTIMATION AFTER REFINEMENT (BEAR): A POST-DOCKING TOOL FOR DRUG DESIGN

Giulio Rastelli

Department of Life Sciences, University of Modena and Reggio Emilia. Via Campi 103, 41125 Modena – Italy

giulio.rastelli@unimore.it

Virtual screening (VS) is a straightforward computational approach for the rapid identification of bioactive compounds from large collections of chemicals. Typically, VS is performed with molecular docking, which is able to computationally screen and rank a large number of molecules into the active site of one or more target structures. However, the inherent limitations in the accuracy of these methods often lead to false positives or false negatives and reduce VS success rates. For this reason, post-docking methods have emerged as a way to improve docking results.

We have developed Binding Estimation After Refinement (BEAR), an automated post-docking tool for the conformational refinement of docking poses through molecular dynamics followed by more accurate binding free energy predictions using MM-PBSA and MM-GBSA. In this talk I will outline the main achievements obtained by using BEAR, with recent examples taken from the design of allosteric inhibitors of protein kinases and multi-target ligands.

Short communication 19

CASE STUDIES FOR SUCCESFUL COMBINATION OF CHOLINESTERASE INHIBITORS AND GPCR LIGANDS WITH PROCOGNITIVE IN VIVO ACTIVITY

Decker Michael,[a] Dolles Dominik,[a] Darras H. Fouad,[a] Drakopoulos Antonios,[a] Strasser Andrea,[b] Wittmann Hans-Joachim,[b] Sotriffer Christoph A.,[a] Pockes Steffen,[c] Sadek Bassem,[c] Maurice Tangui,[d]

[a] *Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Julius Maximilian University of Würzburg, Am Hubland, D-97074 Würzburg, Germany*

[b] *Pharmaceutical and Medicinal Chemistry II, Institute of Pharmacy, University of Regensburg, D-95053 Regensburg, Germany*

[c] *Department of Pharmacology and Therapeutics, United Arab Emirates University, Al Ain, United Arab Emirates*

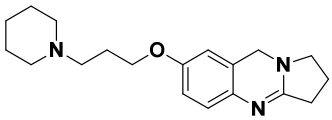
[d] *INSERM UMR-S1198, University of Montpellier, EPHE, F-34095 Montpellier, France*

michael.decker@uni-wuerzburg.de

The combination of cholinesterase inhibitors with GPCR ligands in hybrid molecules seems highly promising for Alzheimer's disease (AD) therapy, since two very different molecular targets can be addressed at the same time. Nevertheless, significant challenges come with this rationale: a) hybrids might possess too high molecular weights to be orally bioavailable and/or pass the blood-brain-barrier, b) the compounds might act in different concentration ranges, c) and selectivity and affinity has to be optimized for several very distinct targets.

We have designed – applying computational methods - and synthesized dual-acting ChE-inhibitors that act with high potency and selectivity also at the histamine 3 receptor (hH$_3$R) [1], and the same could be achieved for cannabinoid 2 receptors (hCB$_2$R) [2, 3], both GPCRs represent important AD targets. Regarding dual-acting ChE inhibitors and hCB$_2$R agonists both covalently connected hybrids using the unselective ChE inhibitor tacrine as well as merged small molecules with high butyrylcholinesterase (BChE) selectivity have been obtained and pharmacologically characterized *in vitro*. Representative examples from all sets of compounds have been investigated *in vivo* in different AD mice models [3].

The case studies demonstrate that it is possible to obtain dual-acting compounds that a) act highly selectively and with high affinity at the respective targets, b) work in the same concentration range ("balanced affinity"), c) exhibit pronounced *in vivo* activity.



Dual-acting AChE inhibitor / hH$_3$ antagonist
- K_i (hH$_3$) = 76.2 nM
- IC_{50}(hAChE) = 33.9 nM
- low molecular weight
- procognitive effects in two *in vivo* memory models

Figure 1: Dual-acting enzyme inhibitor and GPCR ligand

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Short communication 20

IN VITRO, *IN SILICO* AND *IN VIVO* STUDIES OF D2AAK4 AS A POTENTIAL MULTI-TARGET ANTIPSYCHOTIC

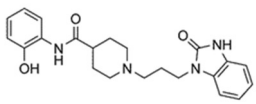
Agnieszka A. Kaczor,[a,b] Andrea G. Silva,[c] Katarzyna M. Targowska-Duda,[d] Grazyna Biala[e], Marian Castro[c]

[a]Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Lab, [d]Department of Biopharmacy and [e]Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy with Division for Medical Analytics, 4A Chodźki St., PL-20059 Lublin, Poland [b]School of Pharmacy, University of Eastern Finland, Yliopistonranta 1, FI-70211 Kuopio, Finland [c]Department of Pharmacology, Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Avda de Barcelona, E-15782 Santiago de Compostela, Spain

agnieszka.kaczor@umlub.pl

The detailed characterization of GPCR ligands has demonstrated that many drugs targeting GPCRs often display a high degree of promiscuity. The ability of GPCR drugs to interact with more than one receptor subtype at low concentrations was first considered a drawback for GPCR-oriented drug discovery. It turned out, however, that the efficacy of certain drugs targeting GPCRs is considered to be mediated by their capacity to regulate several targets at the same time – for instance, in the case of drugs related to the treatment of CNS diseases. In the light of above, 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. The most commonly exploited approach to search for novel antipsychotics is designing compounds with intentional ligand promiscuity resulting in multi-target drugs. Targeting multiple receptors is a recommended approach for designing drugs for complex diseases including schizophrenia. Indeed, in complex psychiatric illnesses, including schizophrenia, selective single-target drugs have been to a great extent a failure.

In search for new potential antipsychotics we identified a novel multi-target ligand of aminergic GPCRs, D2AAK4, using structure-based virtual screening.[1] D2AAK4 possesses nanomolar or low micromolar affinity to D_1, D_2, D_3, 5-HT_{2A} and 5-HT_7 receptors, making it an ideal candidate for a multi-target drug. It has better affinity to 5-HT_{2A} receptor in comparison to D_2 receptor which contributes to its atypicality. Here we present homology modeling, molecular docking and molecular dynamics of D2AAK4 and its molecular targets and animal studies of D2AAK4 as a potential antipsychotic. The main contact of D2AAK4 and all the receptors studied is the electrostatic interaction between the protonatable nitrogen atom of the ligand and the conserved Asp(3.32) as typical for orthosteric ligands of aminergic GPCRs. We demonstrated antipsychotic and, importantly, procognitive properties of D2AAK4 in mouse models.



D2AAK4

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Poster communications

Poster communication 1

CYTOTOXIC ACTIVITY OF DISULFIRAM IN VIRUS -TRANSFORMED ANIMAL CANCER CELLS

Radostina Alexandrova[a], Desislav Dinev[a], Lora Dyakova[b], Zdravka Petrova[a], Tanya Zhivkova[a], Rossen Spasov[c], Abedulkadir Abudalleh[a], Milena Glavcheva[a]

[a]*Department of Pathology, Institute of Experimental Morphology, Pathology and Anthropology with Museum,*
Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, 1113 Sofia, Bulgaria

[b] *Department of Synaptic signalization and communications, Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str.,*

Block 23, 1113 Sofia, Bulgaria

[c]*Department of Pathology, Faculty of Medicine, Sofia University "St.Kliment Ohridski",*

University Hospital Lozenetz,1 Kozyak Str., 1407 Sofia, Bulgaria

rialexandrova@hotmail.com

The dithiocarbamate compound disulfiram (Antabuse) has been used for the treatment of chronic alcohol dependence more than 60 years. The compound has been also reported to expresses promising anticancer activity in both *in vitro* and *in vivo* model systems as well as in human patients. According to the literature available, the influence of disulfiram on the growth of virus-transformed cancer cells is not fully clarified. The aim of the study presented was to evaluate the effect of disulfiram on viability and proliferation of cultured permanent cell lines established from a transplantable chicken hepatoma, induced by the myelocytomatosis virus Mc29 (LSCC-SF-Mc29, the cells express v-myc oncogene) and sarcoma in rat, initiated by Rous sarcoma virus strain Schmidt-Ruppin (LSR-SF-SR, the cells contain v-src oncogene). The investigations were performed by thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay, crystal violet staining, double staining with acridine orange and propidium iodide, hematoxylin and eosin staining in monolayer cultures as well as by 3D-colony forming method. The results obtained revealed that applied at a concentration range of 0.3 –100 µg/ml disulfiram expresses significant cytotoxic and/or cytostatic effects that are time- and concentration- dependent.

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Poster communication 2

Effects of mitochondria-targeted antioxidants in human hepatic cells: a possible therapy for hepatic steatosis?

R. Amorim[1,2], J. Teixeira[1,2], S. Benfeito[1], F. Cagide[1], F. Borges[1], P.J. Oliveira[2]

1 CIQ/ Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal 2 CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

ricardoamorim72@gmail.com;fborges@fc.up.pt; pauloliv@ci.uc.pt

Background: Non-alcoholic steatohepatitis (NASH), one of the deleterious form of non- alcoholic fatty liver disease, remains a major cause of liver-related morbidity and mortality worldwide. Mitochondrial dysfunction plays a key role in the development of NASH. Increased mitochondrial generation of reactive oxygen species (ROS) was measured in NASH animal models, which makes this phenomenon an attractive target for pharmacological and non- pharmacological interventions. In this context, we have generated novel mitochondria-directed antioxidant based on naturally occurring phenolic acids, such as hydroxycinnamic acids (HCA). Our present objective is to investigate whether these novel molecules prevent mitochondrial and cellular damage on an *in vitro* NAFLD model.

Material and Methods: We studied the effects of the novel mitochondriotropic agents (AntiOxCIN$_4$ and AntiOxCIN$_6$) on human hepatoma-derived cell line HepG2 incubated with supra-physiological concentrations of Palmitic acid (PA) or mix of Free Fatty Acids (FFA), measuring cell mass and cytotoxicity, oxidative stress markers, lipid content and mitochondrial activity.

Results: Our *in vitro* lipotoxicity model was considered to be a valid model to study liver steatosis as shown by the increase of lipid accumulation, oxidative stress markers, and cell death. Our data showed that positively charged cations, especially AntiOxCIN$_4$, caused an increase of HepG2 cell mass and induced a transient increase in intracellular ROS without triggering pro-apoptotic responses after 48h of treatment. Chronic treatment with AntiOxCIN$_4$ increased basal respiration and extracellular acidification rates, suggesting an incremental modulation in the energy utilization. AntiOxCIN$_4$ also partly prevented lipid accumulation induced by PA or FFA.

Conclusions: Mitochondriotropic antioxidants based on dietary scaffolds and with complementary antioxidant mechanisms can be used as therapeutic agents in the treatment of oxidative stress-related conditions, such as liver steatosis.

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Poster communication 3

IDENTIFICATION OF *h*ASNS INHIBITORS BY *IN SILICO* DRUG REPURPOSING

Donatella Bagetta,[a] Francesco Ortuso,[a] Stefano Alcaro,[a] Nigel G.J. Richards[b]

[a] *Department of Health Science, University "Magna Græcia" of Catanzaro, Viale Europa, Catanzaro, Italy.*

[b] *School of Chemistry, Cardiff University, Park Palace, Cardiff, Wales, United Kingdom.*

bagetta@unicz.it

The remarkable inverse correlation between leukaemia cell drug therapy sensitivity and their capacity to perform intracellular asparagine biosynthesis provides a rationale for the current widespread use of L-asparaginase (ASNase) in chemotherapeutic protocols for treating childhood acute lymphoblastic leukaemia and some forms of acute myeloblastic leukaemia. Recent studies have demonstrated that the inhibition of human asparagine synthetase (*h*ASNS), that catalyses the biosynthesis of L-asparagine, represents a viable strategy for treating ASNase-resistant leukaemia in the clinic.[1]

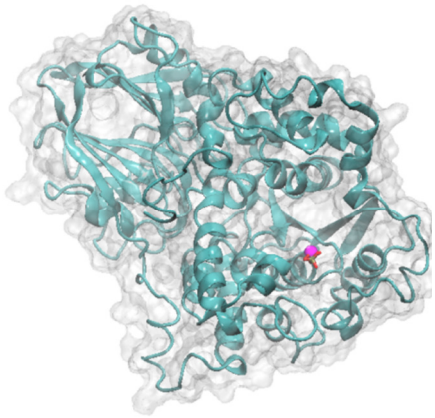

Figure 1: 3D structure of *h*ASNS.

In order to identify new *h*ASNS inhibitors, approved drugs, retrieved from DrugBank[2] database, were virtually screened by a structure-based approach toward the crystal structure of *h*ASNS recently solved by some of us. (Figure 1) In fact, drugs repurposing has the advantage to prevent two of the main failures in drug development: poor toxicological and pharmacokinetic profile. Most promising theoretical complexes between *h*ASNS and screened compounds have been deeply investigated by molecular dynamics simulations. Indeed, anticancer activity of such compounds is already known, but their mechanism of action was unclear. With the aim to validate molecular modeling suggestions, selected compounds will be submitted to experimental evaluation.

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Poster communication 4

NEW SELECTIVE SIGMA-1/HDACi PRODRUGS FOR NEURODEGENERATIVE DISORDERS

Carla Barbaraci, Maria Dichiara, Turnaturi Rita, Fallica Antonino N., Arena Emanuela,

Prezzavento Orazio, Amata Emanuele, Marrazzo Agostino

Department of Drug Sciences, University of Catania , Viale A. Doria 6, 95125 Catania, Italy.

carlabarbaraci@rocketmail.com

Neurodegenerative diseases represent a large group of debilitating conditions with an increased incidence with the aging population. These disorders are characterized by progressive brain and spinal cord damage resulting in a gradual loss of structures and functions of both central and peripheral nervous system. In healthy neurons, a tightly controlled balance between protein acetylation and deacetylation regulates the gene expression and facilitates an adequate neuronal homeostasis. This dynamic equilibrium is maintained through the interplay between histone acetyltransferases (HATs) and deacetylases (HDACs) activities. When an imbalance in favor of histone deacetylation occurs, the aberrant genic transcription results in neurodegenerative disorders. In this view, the use of HDAC inhibitors (HDACi) seems to have neuroprotective effects in both in vivo and in vitro models of brain disorders [1].

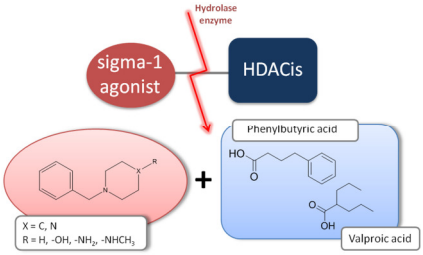

Figure 1: General structure of sigma-1/HDACi prodrugs.

Interestingly, the intracellular membrane associated sigma-1 receptors (σ_1R) are abundantly expressed in neocortex, hippocampus, amygdala, and basal forebrain. A number of data support the use of σ_1R agonists in alleviating deficits in cognitive dysfunction, and providing neuroprotection against amyloid toxicity [2]. In light of the aforementioned, we turned our interest over the combination of well-known HDACi with σ_1R ligands, using a prodrug approach. The novel compounds are made of a portion able to inhibit deacetylase activity, the valproic or phenylbutyric acid, linked to σ_1R agonists, a 4-benzylpiperidine or 1-benzylpiperazine aminic moiety, via an ester or amidic bond which is sensitive to hydrolases. The new synthesized compounds have been evaluated in in vitro σR binding assays revealing one digit nanomolar σ_1R affinity and selectivity over σ_2R. Further studies are in progress to assess biochemical HDACs inhibition and neuroprotective effects.

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Poster communication 5

A Merging Strategy Application: Tacrine-Benzylidenehydrazone Hybrids as Potent ChE Inhibitors with Metal Binding and Neuroprotective Properties

Gülşah BAYRAKTAR,[a] Mümin Alper ERDOĞAN,[b] Sülünay PARLAR,[a] Merve SAYLAM,[a] Güliz ARMAĞAN,[c] Günay YETİK-ANACAK,[d] Vildan ALPTÜZÜN [a]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, IZMIR, TURKEY*

[b] *Department of Physiology, Faculty of Medicine, İzmir Katip Çelebi University, IZMIR, TURKEY*

[c] *Department of Biochemistry, Faculty of Pharmacy, Ege University, IZMIR, TURKEY*

[d] *Department of Pharmacology, Faculty of Pharmacy, Ege University, IZMIR, TURKEY*

gulsah.bayraktar@ege.edu.tr, bayraktargulsah@gmail.com

Alzheimer's disease (AD), the most common form of dementia in the elderly people, is a neurodegenerative disease characterized by various pathologic pathways. Although cholinergic hypothesis relies on the repair of cognitive and memory deteriorations by elevating the reduced acetylcholine (ACh) levels, there are several other pathways in the pathology of AD including formation and accumulation of toxic amyloid-β (Aβ) plaques, neurofibrillary tangles (NFT), oxidative stress, metal ion dyshomeostasis, inflammation, etc [1,2].

There are four approved AChE inhibitors for the symptomatic treatment of AD: Tacrine, donepezil, rivastigmine and galantamine. Although tacrine is no longer used in the treatment of AD due to its hepatotoxicity, it is still a widely used scaffold in the design of Multi Target-Directed Ligands (MTDLs) thanks to its high affinity to AChE [1,2].

Considering multifactorial pathology of neurodegenerative diseases, targeting more than one pathway is emerged as a framework combination of MTLDs in the treatment of AD [2,3]. In this study, we applied merging strategy among these framework combinations to our designed compounds.

In our previous studies, we designed and synthesized hydrazone-containing pyridinium salts and pyridlyne-hydrazone-type compounds with good ChE inhibitory activities [4,5]. On the other hand, there are some examples of hydrazone containing compounds with metal-binding properties in the literature [6]. In the light of all these findings, we chose tacrine as core structure and merged our previously synthesized hydrazone-containing pyridine moieties with tacrine.

In this study, a number of substituted 9-benzylidenehydrazino-1,2,3,4-tetrahydroacridine derivatives were designed and synthesized. Their AChE/BuChE inhibitory activities were evaluated *in vitro* by using Ellman's method [7]. Moreover, their ability to form metal complexes and protective effects of the compounds on H_2O_2-induced oxidative stress in human neuroblastoma cell line (SH- SY5Y cells) were investigated [8,9].

All of the tested compounds exhibited good inhibitory activity against ChE enzymes, found to protect SH-SY5Y cells from H_2O_2-induced toxicity by increasing cell viability and moreover, the compounds were able to form complexes with biometals such as zinc, copper and iron. Among the tested compounds, **G2** was found to have the best inhibitory activity against AChE with the IC_{50} value of 0.088 µM (IC_{50} value of tacrine was found to be 0.076 µM in this study). Similarly, maximum protection against H_2O_2-induced toxicity was observed following 0.1 µM **G2** pretreatment which was resulted in significant increment of cell survival from 44.81% (H_2O_2-only treated cells) to 96.06%.

In conclusion, 9-benzylidenehydrazino-1,2,3,4-tetrahydroacridine core has exhibited promising bioactivity results against AD as a MTDL and further researches are ongoing enthusiastically on this scaffold in our laboratory.

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Poster communication 6

Cu based MOFs: potential candidates in the treatment of Alzheimer's disease

Aleksandra M. Bondžić,[a] Milan V. Senćanski,[a] Marina V. Kirillova,[b] Vânia André,[b] Alexander M. Kirillov,[b] Vesna M. Vasić,[a] Bojan P. Bondzić[c]

[a] *Vinča Institute of Nuclear Sciences, University of Belgrade, PO Box 522, Belgrade, Serbia*

[b] *Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001, Lisbon, Portugal*

[c] *Institute of Chemistry, Metallurgy and Technology, University of Belgrade, Njegoseva 12, Belgrade, Serbia*

aleksandrab@vin.bg.ac.rs

Inhibition of acetylcholinesterase (AChE) represents a promising strategy in the treatment of Alzheimer disease[1] providing inspiration for new discoveries and investigations towards less toxic and more effective potential anti Alzheimer drugs. The aim of our study was to evaluate the effect of newly synthesized three copper(II) MOF derivatives based on different types of Cu_4 units, namely a discrete 0D macrocyclic complex $[O\subset Cu_4\{N(CH_2CH_2O)_3\}_4(BOH)_4][BF_4]_2$, a 1D coordination polymer based on Cu_4 cubane-like units $[Cu_4(\mu_4\text{-}H_2edte)(\mu_5\text{-}H_2edte)(sal)_2]_n\times 7nH_2O$ and a 3D metal-organic framework $[\{Cu_4(Hbes)_4(hba)\}K(H_2O)_4]\times 2H_2O$ on the activity of AChE and to examine possible binding modes of our three compounds to AChE using kinetics measurements and molecular docking approach. Herein we demonstrate that investigated MOFs are capable to effectively inhibit the activity of AChE, in a concentration-dependent manner while $[O\subset Cu_4\{N(CH_2CH_2O)_3\}_4(BOH)_4][BF_4]_2$ has been found to be the most promising agent with IC_{50} values in low µM range. Kinetic measurements revealed reversible and uncompetitive type of inhibition while docking studies pointed out presence of two binding sites.

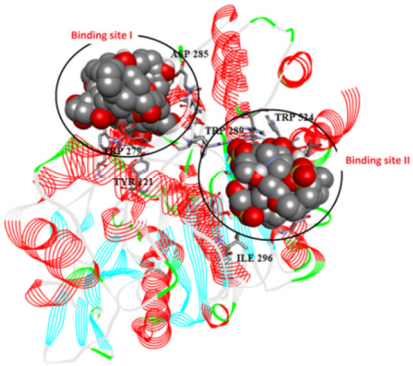

Figure 1: Binding sites of $[O\subset Cu_4\{N(CH_2CH_2O)_3\}_4(BOH)_4][BF_4]_2$ on acetylcholinesterase

The obtained results reveal MOFs as strong and reversible inhibitors of acetylcholinesterase activity which could represent new generation of potential anti Alzheimer drugs.

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Poster communication 7

Chasing Broad Range Dual Inhibitors: Novel Oxindole Inhibitors for Cancer and Alzheimers Disease

Burke, A.J.[a,b] Marques, C.S.,[a] Busto, N.[c], Gaudio, E. [d,e] Bertoni, F.[d], Lopez, Ó.[e]

[a] Centro de Química de Évora, University of Évora, Institute for Research and Advanced Training, Rua Romão Ramalho 59, 7000 Évora, Portugal.

[b] Department of Chemistry, Rua Romão Ramalho 59, 7000 Évora, Portugal,

[c] Department of Chemistry, University of Burgos, Pza. Misael Bañuelos s/n, 09001, Burgos, Spain.

[d] Universita' della Svizzera italiana, Institute of Oncology Research, Via Vela 6, 6500 Bellinzona, Switzerland.

[e] Department of Organic Chemistry. Faculty of Chemistry, Profesor García González, 1. 41012. Seville, Spain.

ajb@uevora.pt

Oxindoles and triazoles are very privileged frameworks in medicinal chemistry and are thus ubiquitous in numerous medicines and natural products.[1] Molecules that contain both these privileged structures are highly desirable from the medical perspective. Two of the most notorious disease categories at the current time are cancer and Alzheimer's disease, which affect a significant portion of the world population, and thus new medicines are required. We have developed an efficient synthetic route that afford libraries of *N*-(1,2,3-triazolmethyl)-3-hydroxy-3-phenyloxindoles starting from cheap biomass derived isatin (Figure 1).[2]

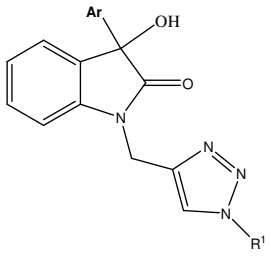

Figure 1: Chiral oxindole-triazole hybrids with anti-cancer and cholinesterase inhibitory properties

The compounds were screened for anti-cancer activity, and showed both cytotoxicity (evaluated by means of the antiproliferative assay MTT test and giving a best IC_{50} of 12mM) and promising anti-proliferative activity for a variety of lymphoma cell lines (with a best value of 19µM).[2b] These compounds have also shown interesting cholinesterase inhibition (Acetylcholinesterase (AchE) and butrylcholinesterase (BuChE)). These results will be discussed in this presentation.

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Poster communication 8

Fine-tuning the neuroprotective and blood-brain barrier permeability profile of dietary based mitochondria-targeted antioxidants

F. Cagide[a], S. Benfeito[a], C. Oliveira[a], C. Fernandes[a], , J. Teixeira[a,b], R. Amorim[a,b], J. Garrido[a,c], C. Martins[d,e], B. Sarmento[d,e,f], F. Remião[g], E. Uriarte[h], P.J. Oliveira[b], F. Borges[a]

[a]*CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal*

[b]*CNC, Center for Neuroscience and Cell Biology, UC-Biotech, University of Coimbra, Cantanhede, Portugal*

[c]*Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic of Porto, Porto, Portugal*

[d]*i3S, Institute of Investigation and Innovation in Health, University of Porto, Porto, Portugal*

[e]*INEB, Institute of Biomedical Engineer, Nanomedicines & Translational Drug Delivery Group, University of Porto, Porto, Portugal*

[f]*CESPU, Institute of Investigation and Advanced Formation in Sciences and Heath Technologies, Gandra, Portugal*

[g]*UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;*

[h]*Applied Chemical Science Institute, Autonomous University of Chile, Santiago de Chile, Chile*

fernandocagide@yahoo.es

Neurodegenerative diseases, such as Alzheimer's disease (AD), are multifactorial age-related diseases deeply associated with oxidative stress and mitochondrial dysfunction. In this context, exogenous antioxidants can be beneficial for decreasing oxidative stress, as they not only compensate the inefficacy of the endogenous defense systems, but also enhance the overall antioxidant response in a pathological condition. Thus, the development of mitochondriotropic antioxidants can be beneficial to prevent/minimize oxidative stress and set up as a novel therapeutic approach.

Along our overarching project related with the design and development of potent and safe mitochondria-targeted antioxidants based on hydroxycinnamic acids (HCAs) and hydroxybenzoic acids (HBAs), novel derivatives with different aromatic substitution patterns, length of alkyl linker between carboxamide and TPP^+ moiety and type of spacer between carboxamide and aromatic ring were obtained. The compounds showed remarkable antioxidant and chelating properties being pyrogallol based systems the most effective. In general, the developed antioxidants presented low cytotoxic effects on human differentiated neuronal (SH-SY5Y) and hepatocarcinoma (HepG2) cells. Overall, the new mitochondriotropic antioxidants exhibited neuroprotective properties on SH-SY5Y cells against 6-hydroxydopamine (6-OHDA) or hydrogen peroxide (H_2O_2) oxidative insults. Moreover, some compounds were able to cross a layer of hCMEC/D3 cells, a blood-brain barrier (BBB) in vitro model, in a time-dependent manner.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145-FEDER-006980, PTDC/DTP-FTO/2433/2014 and NORTE-01-0145-FEDER-000028). F. Cagide, S. Benfeito, C. Oliveira, C. Fernandes, J. Teixeira and R.Amorim grants were also supported by FCT and FEDER/COMPETE and NORTE 2020 funds.

Poster communication 9

Development of piperine-based triphenylphosphonium conjugates as a therapeutic solution for Alzheimer's disease

Daniel Chavarria,[a] Carlos Fernandes, [a] Fernando Cagide,[a] Fernando Remião,[b] Paulo Jorge Oliveira[c] and Fernanda Borges[a]

[a] *CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.*

[b] *UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.*

[c] *CNC – Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech Building, Biocant Park, Cantanhede, Portugal.*

danielfchavarria@gmail.com

Alzheimer's disease (AD) is a progressive and multi-factorial age-related disorder characterized by the loss of memory and cognitive functions[1]. Despite the unclear molecular mechanisms involved in the pathogenesis of AD, numerous targets for potential therapeutics have been identified[2]. These include, among others, the decline of cholinergic transmission and oxidative stress[1,3], as well as mitochondrial dysfunction in particular, a process that precedes the establishment of tau and amyloid beta pathologies[4] and contributes to the synaptic degeneration[5]. Given the multifactorial nature of AD, the modulation of several targets using multitarget directed ligands may enable the desired therapeutic outcome[1]. Therefore, reducing mitochondrial injury may have beneficial effects on neuronal dysfunction and cognitive decline observed in AD patients.

As part of our drug discovery program and following an AD multi-target strategy, novel piperine-based mitochondriotropic antioxidants endowed with cholinesterase inhibitory activity were designed (**Figure 1**).

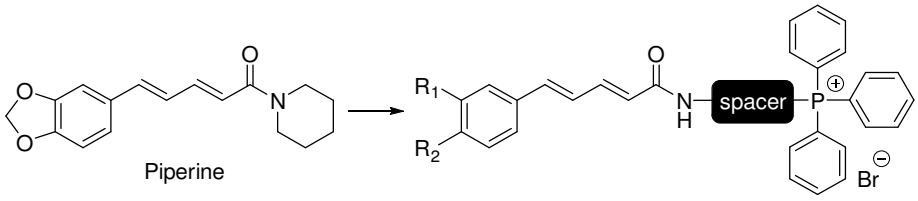

Figure 1: Chemical structure of piperine and derivatives thereof.

Lipophilic triphenylphosphonium conjugates based on piperine were successfully synthesized. The antioxidant profile was assessed using fluorometric and cell-based assays. In addition, the Ellman assay was used to evaluate the acetylcholinesterase and butyrylcholinesterase inhibitory activity and the mechanism of action of the compounds under study. The results obtained so far will be presented in this communication.

Acknowledgements: This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980, and NORTE-01-0145-FEDER-000028). D. Chavarria, C. Fernandes and F. Cagide grants were also supported by FCT, FEDER/COMPETE and NORTE-2020 funds.

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Poster communication 10

In Silico GPCR Polypharmacology

Giovanni Bottegoni, Francesca DeFlorian, Robert T Smith, Juan Carlos Mobarec, Conor CG Scully, Jonathan S Mason, Miles Congreve, Benjamin G Tehan, Chris de Graaf

Heptares Therapeutics, The Steinmetz Building, Granta Park, Great Abington, Cambridge, CB21 6DG

Chris.Degraaf@heptares.com; Giovanni.Bottegoni@heptares.com

Until recently, computer-assisted polypharmacology was an appealing idea, but only occasionally applied.[1] However, in recent years, multiple studies contributed to demonstrating that advanced computational methods can be efficiently rewired for rationally designing compounds endowed with activity at multiple targets. This appears particularly relevant for G protein-coupled receptors, a diverse family of integral membrane proteins involved in the detection of chemical signals. The availability of high resolution GPCR crystal structures solved in Heptares as well as by other companies and academic groups, coupled with an increasing amount of data on ligands and mutants, has raised the relevance of computer-assisted drug design for this important family of receptors to an entirely new level.[2] The application of advanced ligand- and structure-based virtual screening methods, the increasing role of molecular dynamics,[3] and the way these protocols can be efficiently coupled to biophysical techniques[4] will be discussed. Selected examples of how computational design translated into actionable insights for synthesizing compounds with a rationally devised spectrum of activities will be reported.

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Poster communication 11

New therapeutic strategies against clear cell renal cell carcinoma (ccRCC)

Demange Luc,[a] Dufies Maeva,[b] Grytsai Oleksandr,[c] Dumond Aurore,[b] Camara Oumar,[c] Ronco Cyril,[c] Grépin Renaud,[b] Benhida Rachid,[c] Pagès Gilles[b]

[a] *UMR 8638, Faculté de Pharmacie, Université Paris-Descartes, Paris, France.*

[b] *Centre Scientifique de Monaco, Biomedical Department, Principality of Monaco.*

[c.] *Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR 7272, 06108, Nice, France.*

Luc.demange@parisdescartes.fr

Clear cell Renal Cell Carcinoma (ccRCC) affects yearly 200000 new patients worldwide. Its metastatic form is resistant to chemotherapy and radiotherapy, and only 15% of patients survive five years after diagnosis. ccRCC cells present a mutation/inactivation of the von Hippel-Lindau (*VHL*) gene, resulting in the stabilization of Hypoxia-Inducible Factor 1 alpha (HIF-1α), and in the transcription of genes involved in endothelial cells proliferation and Vascular Endothelial Growth Factor A (VEGFA) expression. Consequently, ccRCCs are among the most vascularized tumors, and anti-angiogenic therapeutic strategies are currently givn to patients. However, the response to these drugs are variable. A small fraction of patients benefits for several years but the majority relapses after 1 year maximum. The resistances may be divided into intrinsic resistance (approx. 15% of patients) inducing the sequestration of drugs (*e.g.* sunitinib) in lysosomes,[5] and acquired resistance, due to redundant pro-angiogenic factors, activating both pro-angiogenic pathways and lymphangiogenesis.

Therefore, our team aims at the identification of therapeutic strategies involving original signaling pathway. In particular, we develop triple-action molecules able to concomitantly: i) induce specific anti-proliferative effects on ccRCC cells (effect on ccRCC cells), ii) antagonize both angiogenesis and lyphnagiogenesis (effect on endothelial cells and endothelial growth factors), and iii) strengthens the immune response.

Some of our small-sized organic drugs presented herein exert promising effects in vivo in mice xenografed with human ccRCC cells.

Poster communication 12

Detection of miRNAs Targeting Adiponectin or Leptin Signaling Related Genes in Mammary Tumor Development:

Soner DOGAN[1], Munevver B. CICEKDAL[1], Umit Ozorhan[1], Isin D. EKICI[2], Aysegul KUSKUCU[3], Emre C. Tuysuz, Omer F. BAYRAK[3], Bilge G. TUNA[4]

Departments of Medical Biology[1], Pathology[2], Medical Genetics[3], Biophysics[4],
Yeditepe University School of Medicine, ISTANBUL, TURKEY
soner.dogan@yeditepe.edu.tr

Protective effects of calorie restriction (CR) in many pathophysiologic conditions such as neurogenesis, asthma, diabetes, cardiovascular disease and cancer have been reported. However, the exact molecular mechanism of this phenomenon has yet to be clarified. For this purpose, many molecules and signaling pathways including IGF-I, estrogen, mTOR, adiponectin and leptin signaling related molecules have been studied. On the other hand, small non-coding RNAs called micro RNAs (miRNAs) are the center of many recent studies related to variety of diseases including cancer. The aim of this study was to detect the list of miRNAs which target Adiponectin and/or Leptin signaling related genes in mouse mammary tumor (MT) or mammary fat pad (MFP) of MMTV-TGFalfa mice since roles of Adiponectin and Leptin in breast cancer development have been reported in previous studies. Total of 201 MMTV-TGFα breast cancer model female mice were enrolled into the three different groups; Ad libitum (AL), Chronic CR (CCR) which were applied to 15% of CR compared to AL group and Intermittent CR (ICR) which were applied to 60% CR for one week and consumed AL for the following three weeks in a cyclic manner. Mice were sacrificed at week 10, 49/50 and 81/82, and tissue samples were collected. Chi-square test was used to analyze mammary tumor (MT) incidence rates and grades. For miRNA data analysis ANOVA was used. CCR group had lower MT incidence rates than either AL or ICR groups. For each sample total of 3,195 miRNA was analyzed (ebayes ANOVA, n=3). Using the Transriptome Analysis Console 4.0.1 method, analysis of micro RNAs targeting Adiponectin or leptin signaling related genes in mammary tumor (MT) tissues or healthy mammary fat pad (MFP) tissues from the same animal were compared (n=3). Compared to the healthy MFP tissues, miRNA targeting adiponectin receptor-2 (AdipoR2) gene called "let-7b-5p" was decreased in mammary tumor tissue by two fold at least. This target interaction was validated in mirtarbase and tarbase databases. In addition, Ingenuity Pathway Analysis (IPA) revealed that miR-326-3p targets mRNA of adiponectin gene as predicted target. In this analysis, MT tissue had 2.37 fold less miR-326-3p levels compared to the neighboring healthy MFP tissue in the same animals. On the other hand, when miRNA profiles in healthy MFP tissues of mice with MT bearing mice was compared to the ones without MT development (MT-free), the level of miR-30c-2-3p which targets adiponectin (AdipoQ) gene was 3.59 fold higher in MFP tissue of MT bearing mice compared to the MT free mice. Also, expression of miR-30c-2-3p in MT tissue was 4.96 fold higher compared to healthy MFP tissue of MT free mice. This result was validated by using the mirTarbase database. Moreover, the level of miR-29a-3p which targets leptin (Lep) gene in mouse was 9.91 fold higher in MFP tissue of MT bearing mice compared to the that of MT free mice. This later result was also validated by using the Advaitabio iBioguide database. There were at least two fold differences in these results. In addition, Ingenuity Pathway Analysis (IPA) revealed that miR-500-3p also targets mRNA of adiponectin gene as predicted target. In this analysis, in MFP tissue, MT bearing mice had 7.93 fold higher miR-500-3p levels compared to MT free mice. (Supported by TUBITAK 114S429)

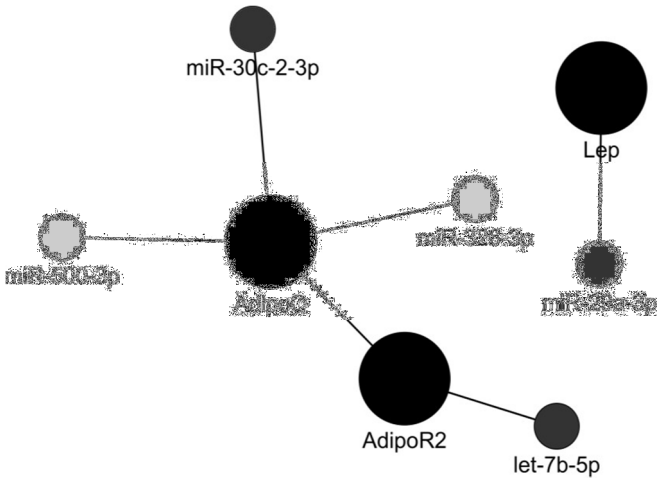

Figure1: Ontology graph of Adiponectin or Leptin signaling related genes targeting miRNAs in MT development. Light, dark grey represent predicted miRNAs while and black color represents validated miRNAs and target mRNAs.

Poster communication 13

Modulating the cytotoxicity of a mitochondriotropic antioxidant by a flexible PEGylation strategy

Carlos Fernandes[a*], Sofia Benfeito[a], Ricardo Amorim[b], José Teixeira[b], Paulo J. Oliveira[b], Fernando Remião[c], Fernanda Borges[a]

[a]*CIQUP-Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007, Porto, Portugal*

[b]*CNC-Center for Neuroscience and Cell Biology, UC-Biotech, University of Coimbra, Biocant Park, Cantanhede 3060-197, Portugal*

[c]*UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal*

**Cevinhas@gmail.com*

Mitochondrial oxidative damage is related to diverse pathologies, including cancer, and neurodegenerative diseases. Shielding mitochondria from oxidative damage with mitochondriotropic antioxidants is by now considered an effective therapeutic strategy.[1] Despite the success of the approach some concerns related with cytotoxicity have been reported. For instance, AntiOxCIN$_6$ is a mitochondriotropic antioxidant based on caffeic acid (CAF) that is cytotoxic in hepatocarcinoma (HepG2) cell lines.[1] PEGylation, often used to enhance drug pharmacologic and pharmaceutical properties,[2] was herein applied to modulate AntiOxCIN$_6$ toxicity drawbacks. So, a dual-functionalization of polyethylene glycol (PEG) with TPP$^+$ and CAF as targeting and antioxidant arms, respectively, was performed by a two-step amidation strategy using ethyl chloroformate and EDC/NHS as coupling reagents. In cellular studies, CPTPP was non-toxic to human HepG2 and neuronal (SH-SY5Y) cells, while both CAF and AntiOxCIN$_6$ demonstrated harmful effects in the same cell lines. Moreover, CPTPP showed remarkable antioxidant effects in cell-based assays against several oxidative stress-induced agents (H_2O_2, t-BHP and FeNTA). From the data it can be concluded that PEGylation technology can modulate the toxicity of mitochondriotropic antioxidants without disturbing the antioxidant profile of the core antioxidant. PEGylation can be considered a relevant tool to hasten the difficulties related to the design and development of mitochondrial nontoxic and operative drug candidates.

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Poster communication 14

Pd(II) complexes with N-heteroaromatic hydrazones as dual targeting DNA/Topoisomerase 1 agents: experimental and *in silico* study

Nenad Filipović,[a,✉] Snežana Bjelogrlić,[b,c] Milan Kojić,[d] Milan V. Senćanski,[e] Milan Nikolić,[f] Aleksandar Višnjevac,[g] Jovana Araškov[f], Marija Miljković[d], Christian Muller[c], Tamara Todorović[f]

[a]*Department of Chemistry and Biochemistry, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*

[b]*National Cancer Research Center of Serbia, Belgrade, Serbia*

[c]*Institut Pluridisciplinaire Hubert Curien, UMR 7178 CNRS Universitée´ de Strasbourg, 67401 Illkirch, France*

[d]*Laboratory for Molecular Microbiology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*

[e]*Center for Multidisciplinary Research, Institute of Nuclear Sciences ‘‘Vinča”, University of Belgrade, Belgrade, Serbia*

[f]*Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*

[g]*Division of Physical Chemistry, Ruđer Bošković Institute, Zagreb, Croatia*

✉ *nenadf.chem@gmail.com*

Anticancer activity of five Pd(II) pyridine-based hydrazone complexes (**1**–**5**, Figure 1) was investigated against a human monocytic leukemia THP-1 (2D) cell line and a breast cancer MCF-7 (2D and 3D) cell line. For complexes with apoptosis as a mechanism of anticancer activity, further investigation revealed that they arrest the cell cycle in G0/G1 phase, induce ROS and *in vitro* inhibit topoisomerase I in a low micromolar range. *In silico* studies corroborate experimental findings and indicate binding to DNA's minor groove. The most active compounds are suitable to be transported *via* blood stream by human serum albumin, as results of CD and fluorescence spectroscopy showed.

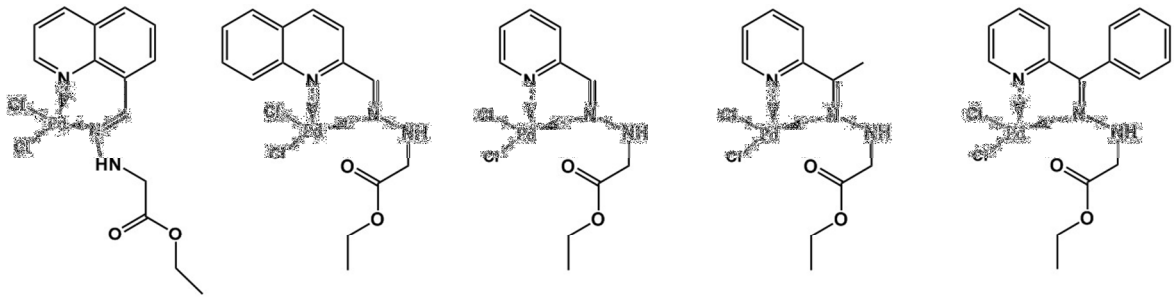

Figure 1: Structures of Pd(II) complexes with N-heteroaromatic hydrazone ligands, derivatives of ethyl hydrazinoacetate and *N*- heteroaromatic carbonyl compounds

Poster communication 15

Could (E)-5-((4-Bromobenzyl)oxy)-2-(4-(3-(piperidin-1-yl) propoxy)benzylidene)-2,3-dihydro-1H-inden-1-one, ST1957, targeting monoamine oxidase B and histamine H3 receptor, be a useful drug to combat neurodegenerative diseases?

Wieslawa Agnieszka Fogel,[a] Anna Stasiak,[a] Stefanie Hagenow,[b] Anna Affini,[b] Holger Stark[b]

[a] *Department of Hormone Biochemistry, Medical University of Lodz, Zeligowskiego 7/9, 90-752 Lodz, Poland*
[b] *Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany*

wieslawa.agnieszka.fogel@umed.lodz.pl

Monoamine oxidase (MAO) B/hH3R dual targeting ligands which combine the H3R pharmacophore with indanone-related MAO B motifs have recently been synthesized as a novel therapeutic approach to combat neurodegeneration associated with amine neurotransmitter signalling deficit (Affini et al. 2018). One compound, ST 1957 which showed very promising results when examined with human recombinant MAOA/B enzymes and human H3R was examined in this study against rat targets to investigate whether its therapeutic efficacy could then be tested in rat model of Parkinson's disease.

The H3R antagonistic activity of ST1957 against the human and rat receptors was examined by binding assays using [3H]N-alpha-methylhistamine and preparations of HEK293 cell membranes expressing hH3R or preparations of rat cerebral cortex. The inhibitory potency of ST1957 (4 x 10-10 to 4 x 10-5M) against human recombinant MAO A and B was analysed by fluorimetry with kynuramine as a substrate while rat cerebral cortex MAO A/B activity was analysed by radiometric assays employing 14C beta-phenylethylamine (MAO B) and 14C serotonin (MAO A). For evaluation of the in vivo effects of ST 1957, the drug doses of 3mg/kg and 15 mg/kg were given intraperitoneally to rats (n=3-4) and the animals sacrificed 2h later. Pargyline (irreversible MAOB inhibitor, 15 mg/kg) was used for comparison.

Antagonistic potency of ST 1957 against H3R as measured by IC50 was 11.2 nM vs 1024 nM for human and rat, respectively, while inhibitory activity of ST1957 against MAO-B and MAO-A were 279 nM and 22.000 nM vs 4520nM and 22,000 nM. Two hours after the drug intraperitoneal administration rat cerebral cortex MAO B was inhibited by 12% and 32% by 5 mg/kg and 15mg/kg ST1957, respectively.

The obtained results indicate that ST1957 i/ has affinities for both human and rat H3R albeit 100 times lower for the rat, ii/ it is also 16 times better inhibitor of human than rat MAO B enzyme, iii/ when peripherally administered to rat it crosses blood-brain barrier and inhibits brain MAO B.

These data warrant further study employing a rat model of Parkinson's disease.

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Poster communication 16

TOWARDS THE MODULATION OF ALZHEIMER'S DISEASE BY BIVALENT LIGANDS: DUAL β-AMYLOID AND TAU AGGREGATION INHIBITORS

Annachiara Gandini,[a,b] Raimon Sabaté,[c] Giuseppe Legname,[b] Maria Laura Bolognesi.[a]

[a] *Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Bologna, Italy;* [b] *Department of Neuroscience, Laboratory of Prion Biology, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy;* [c] *Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain*

annachiara.gandini3@unibo.it

Alzheimer's disease (AD) is the most common cause of dementia. The current lack of effective drugs, with a drug failure rate of 99.6%, has led medicinal chemists to explore new and innovative therapeutic approaches to combat such a complex and multifactorial disorder.[1] Thus, a multi-target drug discovery approach could result as a more promising strategy to provide therapeutic benefits where currently available single-target drugs have failed.[2] β-amyloid (Aβ) plaques and tau protein neurofibrillary tangles represent the two main hallmarks of AD. Therefore, the development of small molecules able to inhibit both protein aggregation, or to disrupt protein aggregates, seems a very promising strategy in AD treatment. In light of this, we designed and synthesized a library of compounds sharing a bivalent structure (**1**-**24**, Figure 1), able to inhibit both Aβ and tau aggregation process. The developed bivalent compounds consist of two protein recognition motifs (PRM) joined by appropriate spacers. As PRM, we selected the 2,4-thiazolidinedione scaffold, while six different lipophilic and aromatic groups were selected as linkers.

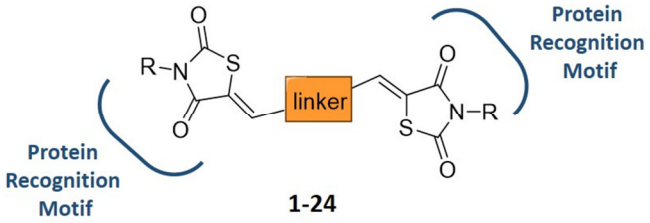

Figure 1: General chemical structure of the bivalent derivatives **1**-**24**.

After the synthesis, the 24 bivalent compounds were tested for their ability to inhibit Aβ and tau aggregation process in intact *Escherichia coli* cells, overexpressing $A\beta_{42}$ and full-length tau. The preliminary results validate the effectiveness of our design strategy, as the compounds demonstrated a promising IC_{50} towards both Aβ and tau protein aggregation inhibition. Selected compounds will be further investigated to gain insights into their binding mode to both Aβ and tau proteins.

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Poster communication 17

REDOX PROPERTIES OF MITOCHONDRIOTROPIC ANTIOXIDANTS: HYDROXYBENZOIC ACID DERIVATIVES

Jorge Garrido,[a,b] Catarina Oliveira,[b] Fernando Cagide [b], E. Manuela Garrido,[a,b] Fernanda Borges[b]

[a] *Department of Chemical Engineering (ISEP), Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 431, Porto, Portugal*

[b] *CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua Campo Alegre, Porto, Portugal*

jjg@isep.ipp.pt

Brain is highly vulnerable to oxidative stress (OS) due to its high-energy demand and oxygen exposure, high level of potent reactive oxygen species (ROS), and low levels of endogenous antioxidants. The redox alterations promoted by OS in specific cellular components lead to a more oxidized state in situ, which is often resultant of the increased production of ROS and/or inadequate intrinsic antioxidant defenses. Therefore, OS have been implicated in the pathogenesis of several age-related diseases.

Hydroxybenzoic acids (HBAc), such as protocatechuic and gallic acids, are widely distributed in plants and fruits being recognized by their diverse biological properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective activities. Hydroxybenzoic acids have been extensively used as a scaffold as part of our group' drug discovery program aimed to develop new antioxidants.[1] In particular, to obtain mitochondriotropic antioxidants HBAc have been covalently bound to a triphenylphosphonium cation (TPP^+) through a carbon aliphatic chain.[2] Some of these derivatives effectively accumulated in rat liver mitochondria, driven by the negative-inside organelle transmembrane electric potential, and prevented lipid peroxidation while exhibiting low toxicity.[2]

During the last decade, electrochemical methods have attracted a great deal of attention given their enormous potential for the assessment of antioxidant capacity. Actually, literature shows that there is a good correlation between electrochemical behavior of compounds and their antioxidant activity. Along our drug discovery project, the redox properties of some mitochondriotropic antioxidants based on HBAc were investigated and the data compared with the antioxidant activity evaluated by total antioxidant assays. The results obtained will be presented in this communication.

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Poster communication 18

A pharmacological screening to improve the anti-lymphoma activity of BET inhibitors

Gaetanina Golino [1], Chiara Tarantelli [1], Filippo Spriano [1], Lorenzo Scalise [1], Alberto Arribas [1], Luciano Cascione [1], Emanuele Zucca [1,2], Anastasios Stathis [2], Francesco Bertoni [1], Eugenio Gaudio [1]

[1] *Università della Svizzera italiana, Institute of Oncology Research, Bellinzona, Switzerland;* [2] *Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.*

Eugenio.gaudio@ior.usi.ch

Introduction. Despite a widespread preclinical anti-proliferative activity of the bromo- and extraterminal domain (BET) inhibitors in lymphomas, the clinical activity in early trials has been limited. Here, we aimed to identify drugs that improve BET inhibition activity performing a pharmacological screening with the BET inhibitor birabresib (OTX015/MK-8628) in combination with a library of 348 compounds in two lymphoma cell lines.

Methods. Two cell lines derived from germinal center B cell (GCB) diffuse large B cell lymphoma (DLBCL) (OCI-LY-19 and WSU-DLCL2) were exposed to birabresib (single dose, 100 nM) in combination with two different doses (20 and 1,000 nM) of 348 compounds. Compounds giving a 1.5-fold decreased proliferation with the combination than with the individual compounds were further evaluated in additional cell lines (the GCB-DLBCL SU-DHL-8 and FARAGE; the mantle cell lymphoma REC1 and the chronic lymphocytic leukemia MEC1) exposed (72h) to increasing doses of birabresib alone and in combination with increasing doses of other compounds. Combinations were validated using another BET inhibitor (CPI-0610). Synergy was assessed with Chou-Talalay combination index (CI): strong synergism (<0.3), synergism (<0.9), additive (0.9-1.1), antagonism/no benefit (> 1.1).

Results. The combinations of birabresib with a series of compounds achieved improved anti-tumor activity than single agents. Besides HDAC and mTOR inhibitors, in accordance with what previously reported by us and others, the ABL/SRC inhibitor dasatinib, the AKT1/2/3 inhibitor MK-2206, the JAK2 inhibitor TG101209 and the LRRK2 inhibitor LRRK2-IN appeared as active combination partners. The screening results were validated in additional four cell lines. The combination of LRKK2-IN with birabresib and with CPI-0610 was synergistic in 6/6 and 5/6 cell lines (no synergism in WSU-DLCL2), respectively. Dasatinib in combination with birabresib or with CPI-0610 was synergistic/strong synergistic in 5/6 cell lines (no synergism in REC1). MK-2206 in combination with OTX015 or with CPI-0610 was synergistic/strong synergistic in 5/6 cell lines. The JAK2 inhibitor TG101209 in combination with OTX015 and with CPI-0610 was synergistic only in 4/6 and 3/6 cell lines respectively.

Conclusion. A chemical screening has identified novel BET inhibitors - containing combinations with anti-tumor activity in lymphoma cell lines, to be further studied.

Work supported by a San Salvatore Foundation grant.

Poster communication 19

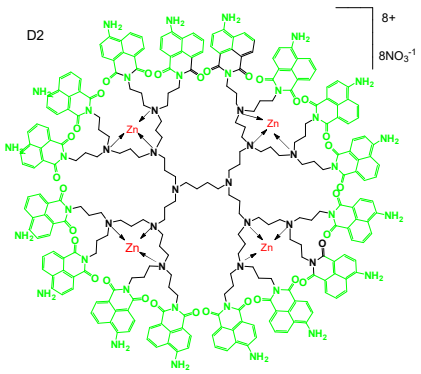
Antibacterial and anticancer activity of Zn(II) poly(propyleneamine) dendrimer complexes, modified with 1,8-naphthalimides

Ivo Grabchev[a], Desislava Staneva[b], Evgenia Vasileva-Tonkova[c], Radostina Alexandrova[d]

[a]*Sofia University "St. Kliment Ohridski", Faculty of Medicine, 1407 Sofia, Bulgaria*
[b]*University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria*
[c]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[d]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 25, Sofia 1113, Bulgaria*

i.grabchev@chem-uni-sofia.bg

Dendrimers with their diverse biological and biomedical activities are a very important part of the research area. Intensive investigations have been carried out especially on their applications as antibacterial and antifungal agents due to the high concentration of surface functional groups in their molecules. New dendrimers with high bioactivity can be obtained by modification with different microbiological monomer agents. Dendrimers are also considered to be an effective tool for anticancer therapy or drug delivery system. Incorporation of metal ions into the dendrimer structure has a new interesting and prospective role in dendrimer chemistry and opens the opportunity to enhance their anticancer activity. Therefore, in the recent years their application in medical chemistry as a new class of metal – containing biomolecules has expanded significantly.



Scheme 1. Zn(II) complex of poly(propyleneamine) dendrimer from third generation modified with 1,8-naphthalimide

In the present study, the antimicrobial growth inhibition of *B. subtilis, P. aeruginosa* and *C. lipolytica* pathogens and anticancer activity against different tumor cell as model systems: HeLa; LSCC-SF-Mc29 and E7 and Lep-3 of poly(propyleneamine) dendrimers from first and third generations, modified with 1,8-naphthalimide and their Zn(II) complexes have been investigated and discussed. The dendrimers were deposited onto the surface of a 100% cotton fabric and their microbiological activity was also investigated and discussed with regard to potential applications of those textiles as antibacterial materials.

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Poster communication 20

A multi- targeting approach to discover new molecules to inhibit the 20s proteasome in anticancer therapy: computer- aided drug design methodologies and biological evaluation

[a,b] Romina A Guedes, [a]Carlota Leonardo Sousa, [c] Andre Falcao, [d] Eleonora Gallerani, [d] Riccardo Gavioli, [a] Andreia Neves Carvalho, [a]Maria Joao Gama, [a,b] Jorge AR Salvador, [a] Rita C Guedes

[a] *iMed.ULisboa, Faculty of Farmacy, University of Lisbon, Av. Professor Gama Pinto. 1649-003 Lisboa, Portugal*
[b] *Center for Neurosciences and Cell Biology, University of Coimbra. Rua Larga – Faculdade de Medicina, 3004-504 Coimbra, Portugal*
[c] *Department of Informatics, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal*
[d] *University of Ferrara. Department of life sciences and biotechnology. Via Fossato di Mortara 64A, 44121 - Ferrara, Italia*
[e] *Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal*

rguedes@ff.ulisboa.pt

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

This work relies on a computational-based drug discovery approach to find alternative new, selective (and more effective) small molecules as 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 techniques (i.e. molecular docking, pharmacophore modeling, structure-based virtual screening and molecular descriptors calculation) in order to identify potential hit compounds. The selected compounds were tested in cell growth inhibition assays, being also performed inhibition assays for the chymotrypsin-like, trypsin-like and caspase-like activities of the proteasome using fluorogenic substrates.

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Poster communication 21

NOVEL HYBRIDS OF NEUROPROTECTIVE NATURAL PRODUCTS: *IN VITRO* INVESTIGATIONS REVEAL OVERADDITIVE EFFECT

Sandra Gunesch[a*],, Simon Schramm[a],_ Florian Lang[b],_ Judit Roa[c],_ Petra Högger[b],_ Raimon Sabaté[c],
Pamela Maher[d] and Michael Decker*[a]

[a] *Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Julius Maximilian University Würzburg, Am Hubland, D-97074 Würzburg, Germany*

[b] *Clinical Pharmacy, Institute of Pharmacy and Food Chemistry, Julius Maximilian University Würzburg, Am Hubland, D-97074 Würzburg, Germany*

[c] *Department of Pharmacy and Pharmaceutical Technology and Physical-Chemistry, School of Pharmacy and Institute of Nanoscience and Nanotechnology (IN2UB), University of Barcelona, 645 Diagonal Ave, E-08028 Barcelona, Spain*

[d] *The Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037, USA*

michael.decker@uni-wuerzburg.de

Natural products hold considerable interest for the development of novel neuroprotectants [1] in part but not exclusively due to their antioxidant properties counteracting neurotoxic oxidative stress, one of the main hallmarks of neurodegenerative diseases. The flavonolignan silibinin and naturally occurring phenolic acids, like cinnamic and ferulic acids, were covalently connected to hybrids and their physicochemical antioxidant radical-scavenging properties as well as their *in vitro* neuroprotectivity profile were investigated. [2] Since the relevance of any single cellular screening assay can be questioned based on the cell type or the nature of the toxic insult [3], a phenotypic screening approach combining multiple assays was used to investigate *in vitro* activities. Despite weak activities in the physicochemical FRAP assay, silibinin esters of α,β-unsaturated phenolic acids showed pronounced overadditive neuroprotective effects in the oxytosis assay using murine hippocampal neuronal cells (HT-22). Already at low concentrations the hybrids greatly exceeded the effects of equimolar mixtures of silibinin and the respective acids in the neuroprotection assay. In the course of phenotypic screening assays, hybrids of cinnamic and ferulic acids exhibited overadditive effects regarding inhibition of microglial activation, PC12 cell differentiation and *in vitro* ischemia. Furthermore, anti-aggregating abilities of the hybrids against Aβ42 peptide and τ protein were higher compared to their respective components. The results demonstrate that non-toxic natural antioxidants connected as esters with medium-term metabolic stability exhibit very pronounced overadditive effects in a portfolio of biological assays. The overadditive effects in several phenotypic assays underlines the importance of natural product hybrids as a multitarget approach to combat complex and multifactorial diseases like AD.

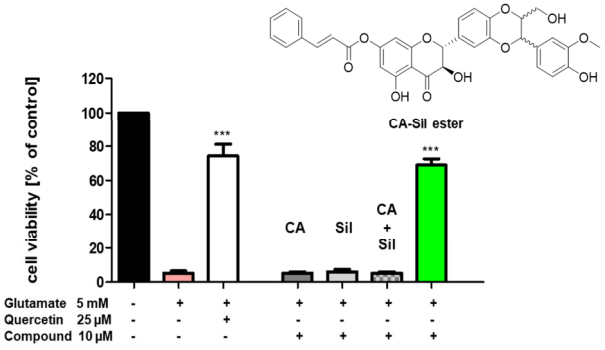

Figure 1: Overadditive neuroprotective effect of 7-*O*-cinnamoyl silibinin (green) compared components cinnamic acid (CA) and silibinin (Sil) as well as their equimolar mixture.

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Poster communication 22

Design and Synthesis of TKIs Specifically Interacting with SBCP (VEGFR2 TK)

Peter Šramel,[a] Miroslav Murár,[a] Gilles Hanquet[b] and Andrej Boháč[a,c]

[a] *Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina, 842 15 Bratislava, Slovakia*

[b] *Université de Strasbourg, Ecole Européenne de Chimie, Polyméres et Metériaux (ECPM), Laboratoire d'innovation moléculaire appliquée (UMR CNRS 7042), 25 rue Becquerel, 67087 Strasbourg Cedex 2, France*

[c] *Biomagi, Ltd., Mamateyova 26, 851 04, Bratislava, Slovakia*

sramel.peter@gmail.com

Twenty-two derivates of *N*-(5-(ethylsulphonyl)-2-methoxyphenyl)-5-phenyloxazol-2-amines (**AAZ** (**1**) included)[1] with determined enzymatic (**IC_{50}, VEGFR2**) and cellular activities (**IC_{50}, hu-HUVEC/VEGF**) were described. Only five of them were substituted on an oxazole joint phenyl ring in "*para*" position (4-Cl, 4-CN, 4-$CONH_2$, 4-OMe and 4-F).[2] All *para* substituents are projected towards a small **Salt Bridge Containing Pocket (SBCP)** that we discovered recently in a special DFG-IN/OUT kinase conformation. The SBCP pocket (consisting from Lys866, Glu883 and Phe1045 amino acid residues) represents an important interaction region over the ATP-binding site in VEGFR2 TK. No discussion about the interactions with this pocket was noted in the literature. (Figure 1)

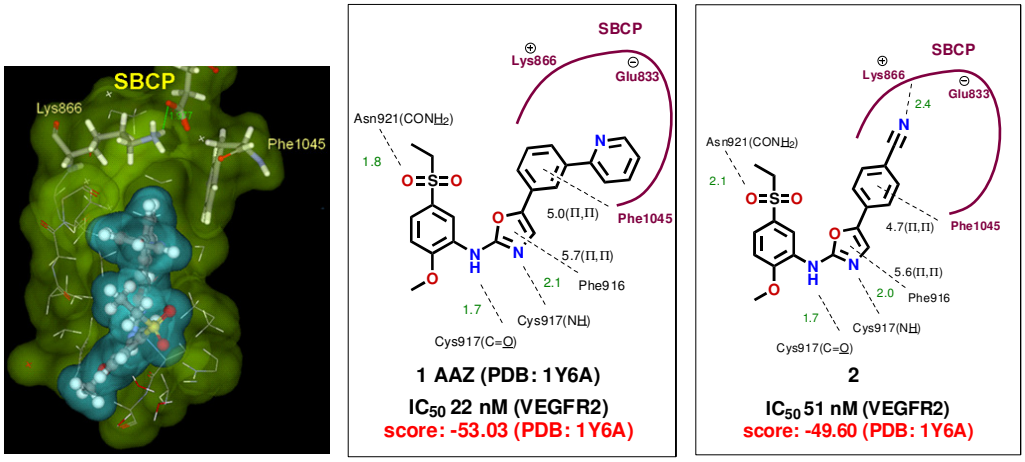

Figure 1 The left picture represents 3D visualization of AAZ (1) in VEGFR2 TK binding place with highlighted SBCP arrangement. The second and third picture represent binding interaction maps of 1 (AAZ) and one predicted for the previously prepared para substituted derivative (2) with SBCP domain.

Our project is focused on a development of novel "*para*" substituted **AAZ**-based inhibitors (**3**, **4**, **5**, **6**) possessing predicted synergy between the specific SBCP interaction and favorable interactions of pyrid-2-yl pharmacophore known from **1** (**AAZ**). We also designed two novel *"meta"* substituted compounds (**7**, **8**) containing imidazol-2-yl, imidazol-4-yl pharmacophore predicted to interact in their protonised form with Phe1045 from SBCP. (Figure 2)

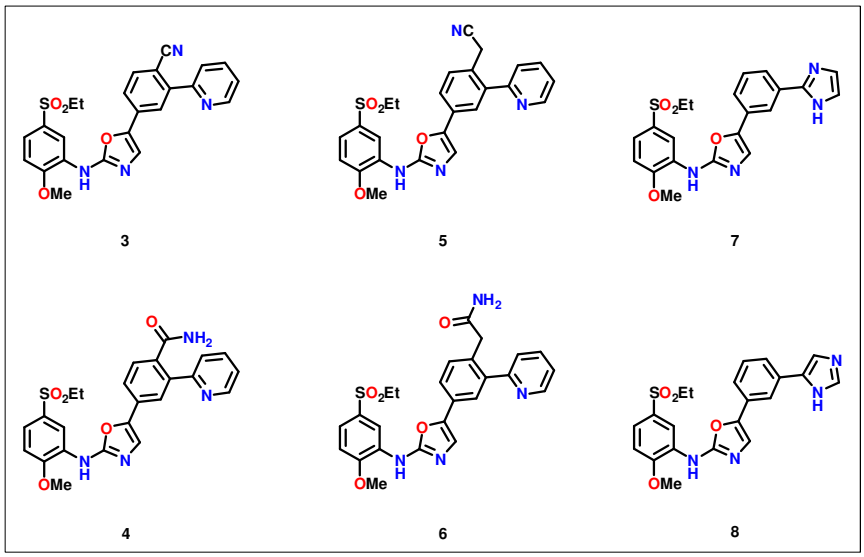

Figure 2 The designed AAZ-based TKIs (3, 4, 5, 6, 7, 8).

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Acknowledgements

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Poster communication 23

Synthesis and biological activity of predicted ALR2 inhibitors

M. Hlaváč,[1,3] P. Šramel,[1,3] L. Kováčiková,[2] G. Hanquet,[3] M. Májeková,[2] M. Štefek,[2] A. Boháč[1,4]

hlavac.matus91@gmail.com, sramel.peter@gmail.com

[1] *Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina, 842 15, Bratislava, Slovakia,*
[2] *Institute of Experimental Pharmacology and Toxicology, SAS, Dúbravská cesta 6, 841 04, Bratislava, Slovakia*
[3] *(ECPM), Laboratoire de Synthése et Catalyse (UMR CNRS 7509), Université de Strasbourg, Ecole Européenne de Chimie, 25 rue Becquerel, 67087 Strasbourg cedex 2, France*

[4] *Biomagi, Ldt., Mamateyova 26, 851 04, Bratislava, Slovakia*

Inhibition of an aldose reductase (ALR2), the first enzyme of a polyol pathway, is a promising approach in the treatment of late diabetic complications. Several of ALR2 inhibitors contain an important carboxylic group, which interacts with an anion binding pocket in an active site of ALR2. Recently, 2-(3-thioxo-2*H*-[1,2,4]triazino[5,6-*b*]indol-5(3*H*)-yl)acetic acid (**CMTI or cemtirestat**) was identified as a powerful ALR2 inhibitor possessing a good ALR2 / ALR1 selectivity and drug-like properties.[1] Based on the structure drug design, several potential analogues of **CMTI** were proposed. Among them, compound **1 (OCMTI,** IC_{50} = 42 nM**)** showed almost 3-fold higher inhibitory activity in an *in vitro* ALR2 enzymatic assay and more as 8-fold higher selectivity relative to ALR1 (IC_{50} >100 µM) than **CMTI (1)** (IC_{50} = 116 nM ALR2 and 35.10 µM ALR1). Based on these results we can conclude, that isosteric replacement of sulphur with oxygen plays an important role in the inhibition of ALR2 and its selectivity.

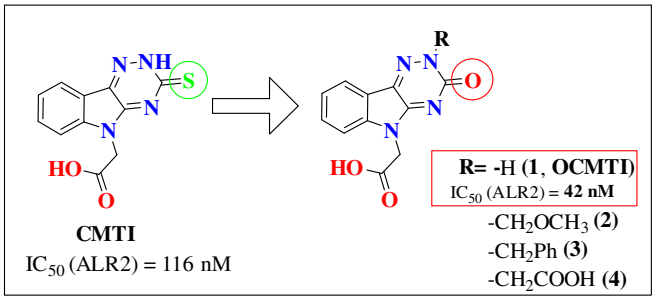

Figure 3. The structures of CMTI, its oxygen analogue derivatives 1-4 and some of determined ALR2 IC_{50} activities.

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Poster communication 24

REVERSAL ANTIBIOTIC RESISTANCE with MTDL STRATEGIES: NMP (NAPHTHYLMETHYLPIPERAZINE) HYBRIDS

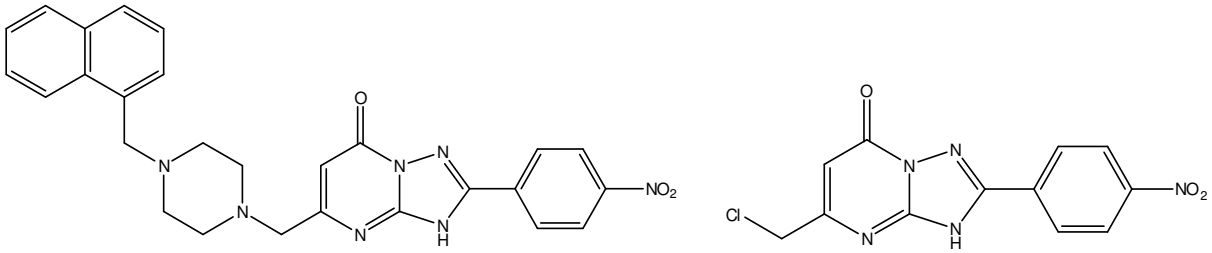
Huseyin Istanbullu[a], Gulsah Bayraktar[b], Ismail Ozturk[c], Gunes Coban[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, Izmir, Turkey*
[c]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey*

huseyin.istanbullu@ikc.edu.tr

Infections caused by resistant microorganisms lead to serious problems, especially in susceptible patient groups such as children, elderly, and immunosuppressed patients (1). One of the resistance pathways is extrusion of the drugs before it reaches its target by EP (Efflux Pump) and overexpression of these pumps (2).

The aim of this study is to obtain novel hybrid compounds with triazolopyrimidine ring, provides antimicrobial activity, bearing NMP moiety as an EP inhibitor. The antimicrobial activity of obtained compounds was tested by disk diffusion and microdilution methods against Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive *(Staphylococcus aureus* ATCC 29213/25923 and *Enterococcus faecalis* ATCC 29212) bacteria. Gentamicin was used as control group of antibiotic. Additionally, the EP inhibition of the compounds was investigated using EtBr (Ethidium Bromide) assay (3).



H5 **TP5**

Figure 1. A sample of synthesized compounds (H5) and triazolopyrimidine precursor (TP5)

Among the tested hybrid compound H5 (3*H*-5-((4-(naphtahelene-1-ylmethyl)piperazin-1-yl)methyl)-2-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7-one) showed promising activity against *E. faecalis, S. aureus* and *E. coli.* Antimicrobial activity studies against resistant isolates from clinics, and the molecular biological studies on the expression of the EP genes of bacteria are in progress enthusiastically at our lab.

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Poster communication 25

Expression of activator protein-1 (AP-1) family members in breast cancer: association of c-FOS/MACC1 and FosB expression with a well-differentiated, receptor-positive tumor phenotype

Daria Ler[1,2], Jasminka Mujic[1,2], Karin Milde-Langosch[3], Volkmar Mueller[3]

[1]Genetics and Bioengineering, International University of Sarajevo, Hrasnicka cesta 15, Sarajevo, Bosnia and Herzegovina

[2]Molecular Diagnostics, Eurofarm Centar polyclinic, Kolodvorska 5, Sarajevo, Bosnia and Herzegovina

[3]Department of Gynecology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, Hamburg, Germany

daria.ler@eurofarm.ba

In previous report we have shown that higher MACC1 expression in breast cancer patients correlates with poor prognosis and shorter disease free survival but probably depends on different clinical futures such as metastases, tumor grade and stage, ER- and PR receptor. Up to date, there are a small number of articles about the promoter of MACC1 gene and its transcriptional regulation. Manisha J. *et al* has reported the possible promoter regulation of MACC1 by using promoter luciferase that directs the transcription of MACC1. They have identified binding sites for well know transcription factors involved in tumorigenesis and cell growth such as AP-1, Sp-1 and C/EBP expression and activity that are seen in many tumor types [1]. The AP-1 family consists of dimeric complexes of either homodimers of Jun family members (c-Jun, JunB and JunD) or heterodimers of Jun or Fos family members (c-Fos, FosB, Fra-1 and Fra-2). AP-1 proteins are involved in the regulation of a variety of cellular processes including proliferation, differentiation, growth, apoptosis, cell migration, and transformation [2]. The AP-1 is an oncogenic transcription factor found to be overexpressed in many cancer types demonstrating promising therapeutic targets. Additionally, AP-1 has been shown to promote proliferation of ER-positive breast cancer cells (i.e. MCF-7), where up-regulated AP-1 activity has been associated with tamoxifen resistance and increased invasiveness [3]. However, to our knowledge there is no information about the correlation of MACC1 expression and AP-1 proteins in breast cancer. In this study we report a strong correlation between c-FOS and MACC1 expression in ER+ receptor patients, which points to potential involvement of these markers in breast cancer genesis. Furthermore, a significant correlation was observed between FosB expression in group of breast cancer patients with lymph node negative (p=0.03) and progesterone- and estrogen positive status. These findings potentially indicate a significant role of Fos B in early stage of breast cancer. Finally, it is important to recognize a prognostic/predictive biomarker which could identify and stratify patients in subgroups that could most benefit from certain therapy and to avoid the use of chemotherapy for patients with estrogen receptor (ER)-positive breast cancer.

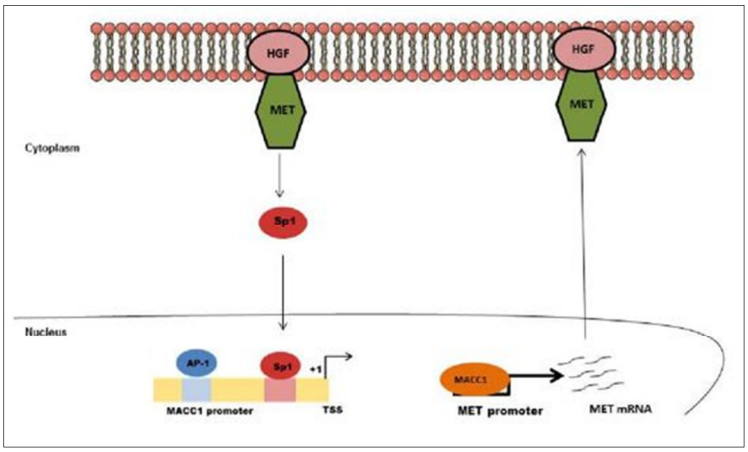

Figure 1: Schematic representation of MACC1 and AP-1 regulation.

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Poster communication 26

EVALUATION OF THE PROTECTIVE EFFECTS OF Mn-*SALEN* DERIVATIVES ON H_2O_2-INDUCED OXIDATIVE STRESS IN SH-SY5Y NEUROBLASTOMA CELLS

Marcelino Maneiro,[a] Lara Rouco,[a] Ana M. González-Noya,[a] Eva Alonso,[b] Rebeca Alvariño,[b] Luis M. Botana[b]

[a] *Dept. de Química Inorgánica, Facultade de Ciencias, Campus Terra, University of Santiago de Compostela, Lugo, Spain.*

[b] *Dept. de Farmacología, Facultade de Veterinaria, Campus Terra, University of Santiago de Compostela, Lugo, Spain.*

marcelino.maneiro@usc.es

Oxidative stress is the effect of the imbalance between antioxidants and oxidants where the cellular defenses are not able to neutralize ROS (Reactive Oxygen Species) overproduction. In human cells, oxidative stress is involved in several pathologies such as cancer, ageing, or neurodegenerative diseases, particularly Alzheimer disease.[1] Manganese complexes with *salen* and related ligands as *salpn* or *salophen*, which contain different spacers between the aromatic rings, emerge as effective ROS scavengers in different antioxidant tests.[2] Hence, we have recently developed a type of complexes which are capable to act as selective $^{\bullet}OH$ scavengers. Their superoxide dismutase activity was also evaluated through the compounds ability to compete with ferricytochrome c for the superoxide radical anion generated by the xanthine/xanthine oxidase (XO) system.

In our present work, we examined for the first time the neuroprotective effects of this type of complexes against oxidative stress in a human neuronal model. SH-SY5Y cells were coincubated with Mn-*salen* derivatives at concentrations ranging from 0.001 to 10 µM, and the well-known oxidant hydrogen peroxide at 75 µM for 6 h, and the protective effects of the compounds were evaluated. Among the complexes tested, those complexes incorporating the ancillary ligand dicyanamide were the most promising compounds, improving mitochondrial function and decreasing reactive oxygen species levels in human neuroblastoma cells treated with the compound at 0.001, 0.01, 0.1 and 1 µM. Among the complexes studied it is noteworthy to mention that those most active complexes in the SOD xanthine/xanthine oxidase test are not the ones that exhibit better protective effects on SH-SY5Y neuroblastoma cells since the ability to transfer the cell membrane arises as the key issue to develop this protective action.

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Poster communication 27

DEVELOPMENT OF NOVEL THERAPEUTIC THAT CAN TARGET MULTIPLE RECEPTORS FOR TREATMENT OF PARKINSON'S DISEASE

Samman Mansoor,[a] Gulru Kayik,[b] Essam Alshahaby[c], Mustafa Guzel,[c] Serdar Durdagi [b] and Ozge Sensoy[a]

[a] *The School of Engineering and Natural Sciences, Istanbul Medipol University, Istanbul, Turkey*

[b] *Computational Biology and Molecular Simulations Laboratory, Department of Biophysics, School of Medicine, Bahcesehir University, Istanbul, Turkey*

[c] *International School of Medicine, Istanbul Medipol University, Istanbul, Turkey*

smansoor@st.medipol.edu.tr

Parkinson's disease (PD), which is the second most common neurodegenerative disorder, is caused by disruption of cells which provide dopamine to the striatum in the brain. Therefore, the main approach made for treatment of the disease has based on increasing dopaminergic signaling by a) using dopamine agonists, b) preventing dopamine breakdown via monoamine oxidase (MAO) enzymes or c) supplying additional dopamine in the form of L-dopa. Even though L-dopa is known as the most effective drug so far, its efficacy decreases with time due to the use of high dosage of the drug. Moreover, it also causes motor complications such as motor fluctuations and dyskinesia. In this multidisciplinary project, we have aimed for developing hetero-bivalent ligands that target A_2AR (Adenosine 2A receptor)-D_2R (Dopamine 2 receptor) hetero-tetramer, which has been shown to be the dominant form of A_2AR-D_2R dimer[1]. Firstly, we design and dock hetero-bivalent ligands to the receptors and investigate the molecular mechanism of allosteric interactions within the hetero-tetramer by means of accelerated molecular dynamics (MD) simulations. Subsequently, successful drug candidates are synthesized and tested *in vitro* for their activities. More importantly, the drug candidates are also tested by in silico and in vitro models for their permeation against blood-brain barrier. So far, hetero-bivalent ligands were only used as molecular tools for detecting the existence of the receptor dimers. The results of the current study will provide a chance to test the capability of hetero-bivalent ligands for being used as therapeutic molecules. Finally, successful candidates will be tested in vivo Parkinson's disease models as shown in Figure 1. In this way, we expect to develop more effective therapeutic molecules to alleviate the symptoms of Parkinson's disease hence increasing the quality of patient's life.

Figure 1: Work plan of the multidisciplinary project

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Poster communication 28

Design and Development of Dual -Target Ligands Based on Benzopyran for the Treatment of Parkinson's Disease

F. Mesiti,[1,2*] A. Gaspar[1], D. Chavarria[1], T. Silva[1], S. Alcaro[2], F. Borges[1]

francesco.mesiti@hotmail.it

[1]CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal;

[2] Department of "Scienze della Vita", University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy

Parkinson's disease (PD) is an age-related neurodegenerative illness characterized by four cardinal motor features (bradykinaesia, rigidity, resting tremor and postural instability) and several non-motor symptoms[1]. Although the depletion of dopamine is described as the key neurochemical impairment in PD, significant deficits in cholinergic transmission have been associated with the cognitive decline and gait dysfunction [1].

Currently, the clinical management of PD is symptomatic and mainly based on restoring dopamine levels[2]. However, as a significant deficit in cortical acetylcholinesterase activity has been described it was hypothesized that AChE inhibitors can be beneficial to manage the non-motors symptoms associated to the disease. Rivastigmine has been licensed by FDA for the treatment of patients with mild to moderately severe dementia associated with PD, although several lines of evidence have also pointed out clinical benefits for donepezil and galantamine[1].

Taken into account PD multifactorial nature and the need of disease-modifying drugs our group started a project focused on the design and development of dual-target-directed ligands aimed to restore dopamine and cholinergic levels. As chromone and coumarin scaffolds were validated as MAO-B or AchE inhibitors[4-7] the first step of our project was aimed to identify the key structural of benzopyrone ring important for each activity and attain novel hybrid chemical templates which can be of great value in surmounting the paucity of effective disease-modifying agents in the pipeline of PD. The results obtained so far will be presented in this communication.

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Poster communication 29

1,6 NAPHTHYRIDINES REPURPOSING: FROM ANTIPROLIFERATIVE TO ANTIVIRAL

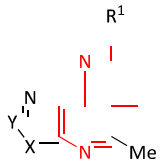
Ilaria Frasson,[a] Virginia Spanò[b], Anna Carbone[b] Sara N. Richter[a], Paola Barraja[b], Alessandra Montalbano[b]

[a] *Dipartimento di Medicina Molecolare, Università degli Studi di Padova, Via Gabelli 63, 35121 Padova (Italy).*

[b] *Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo (Italy).*

alessandra.montalbano@unipa.it

Herpes simplex-1 (HSV-1) is a human pathogen that infects the majority of the worldwide population. HSV-1 establishes a lifelong infection within the host neurons, reactivating upon stressful stimuli. HSV-1 has been associated to cancer pathology, neuropathology and has become clinically important in immunocompromised patients. Moreover, HSV-mediated stromal keratitis is the leading cause of infectious corneal blindness in developed countries. Current clinical therapies rely on nucleoside drugs such as acyclovir to ameliorate primary infections and reduce the symptoms of reactivations. These treatments do not fully suppress viral shedding and long term therapy has led to the development of drug-resistant strains, representing a novel worldwide threat[1]. Therefore, it has become mandatory to explore non-nucleoside cores to develop innovative antiherpetic agents, displaying the lowest cytotoxic effect.
In the last years, our efforts aimed at the study of heterocyclic scaffod as precursor of new potential photosensitizers[2]. We have recently synthesized [1,2,3]triazolo[4,5-*h*][1,6]naphthyridines (**1**) and [1,3]oxazolo[5,4-*h*][1,6]naphthyridines (**2**) endowed with promising singlet oxygen sensitizer properties. Crucially, the antiproliferative assays proved that all compounds, belonging to both classes displayed no cytotoxic effect on human cell lines without UV exposure.



1 X=N-R; Y=N **2** X=O; Y=C-R

Since 1,6-naphthyridines have been recently proposed as a novel class of anti-HSV agents by other groups[3,4], we are about to test the potentialities of our tricyclic systems, undertaking a repositioning study as antiviral agents. The effect on the viral cycle will be assessed on both wild type and mutant HSV-1 viruses, with characterization of the viral step hindered by the most active compounds. Moreover, the antiviral effect of tested compounds will be checked also on HSV-1 strains isolated from patients by the Microbiology and Virology Unit of Padova Teaching Hospital.

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Poster communication 30

CYCLOPENTENEDIONES AS NEW BIOACTIVE LIGANDS

David Novák, Jan Vacek

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

david.novak@upol.cz

Cyclopentenediones (CPDs) are secondary metabolites of higher plants, fungi, algae, cyanobacteria and bacteria. A common denominator of CPDs is the cyclopent-4-ene-1,3-dione skeleton (Fig. 1), which is modified by several functional groups. The heterogeneity of these substitutions is reflected in around one hundred CPDs reported to date. Most of the derivatives were isolated primarily from plant sources. Synthetic analogues were then prepared with new biological activities and more interesting pharmacological potential. Antifungal substances called coruscanones are the most studied of the CPDs. Other intensely investigated CPDs include lucidone, linderone, asterredione, involutone, nostotrebin 6, TX-1123, G2201-C, madindolines and many others. In addition to antibacterial and antifungal effects, a broad spectrum of biological activities for CPDs has been reported in the past two decades, especially anti-inflammatory, cytostatic and specific enzyme inhibitory activities. The CPD skeleton has been identified in a number of substances isolated from the plant kingdom; hence, CPDs can be referred to as a new group of natural bioactive substances. The main goal of this contribution is to define CPDs with respect to basic chemistry, isolation, synthetic approaches and description of their biological effects. Special attention is given to a detailed view into biological activities of CPDs *in vitro* and their phamacological potential.

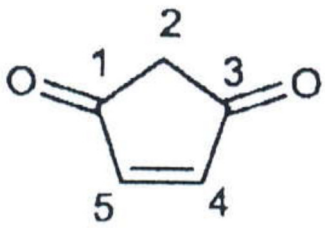

Figure 1: General structure of CPDs.

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Poster communication 31

Exploring African Medicinal Plants for Drug Discovery: Databases Resources and Lead Compound Discovery

Fidele Ntie-Kang,[a,b]

[a] *Department of Informatics and Chemistry, University of Chemistry and Technology Prague, Technická 5 166 28 Prague 6 - Dejvice, The Czech Republic*

[b] *Department of Chemistry, University of Buea, P.O. Box 63 Buea, South West Region, Cameroon*

ntiekfidele@gmail.com

There seems to be a recent interest in natural product-based discovery,[1] as increasingly new tools are being developed in order to accelerate natural product dereplication and lead discovery, assisted by molecular modeling. This talk plans to focus on new natural product database tools and datasets for the discovery of lead compounds from African floral matter. Prior to the investigations, data regarding compounds which had been identified from the aforementioned sources were scattered in literature sources, some of which were inaccessible to the wider community of scientists. Data was collected on the constituent metabolites, their biological activities, as well as the uses of the source organisms in traditional medicine, which have been made available via the web. Moreover, the investigations have led to the identification (assisted and non assisted by molecular modeling) of lead compounds with anti-HIV, anti-*Onchocerca* and sirtuin inhibitory properties (Fig. 1),[2] beginning from plants with popular uses in African traditional medicine.

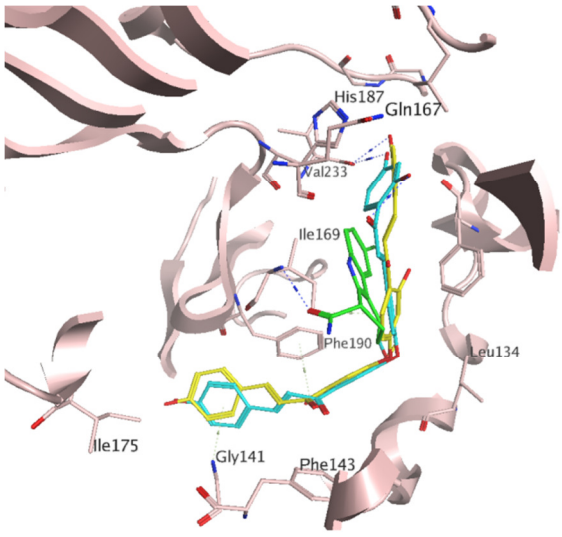

Figure 1: Predicted binding mode of active compounds in the peptide binding pockets of sirtuin2 (PDB ID: 4R8M).

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Poster communication 32

Hydroxybenzoic Acid Derivatives as Dual-Target Ligands: Mitochondriotropic Antioxidants and Cholinesterase Inhibitors

Catarina Oliveira,[a] Fernando Cagide,[a] José Teixeira,[a,b] Ricardo Amorim,[a,b] Tiago Silva,[a] Jorge Garrido,[a,c] Fernando Remião,[d] Santiago Vilar,[e] Eugenio Uriarte,[e,f] Paulo J. Oliveira,[b] Fernanda Borges[a]

[a] *CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal;*

[b] *CNC – Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech, Biocant Park, Cantanhede 3060-197, Portugal;*

[c] *Department of Chemical Engineering, Superior Institute of Engineering of Porto (ISEP), IPP, Rua Dr. António Bernardino de Almeida, 431, 4200-072, Porto, Portugal;*

[d] *UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;*

[e] *Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago Compostela, Praza Seminario de Estudos Galegos, s/n. Campus sur, 15782, Santiago de Compostela, Spain;*

[f] *Applied Chemical Science Institute, Universidad Autonoma de Chile, 7500912, Santiago de Chile, Chile.*

agoliveira@fc.up.pt

Neurodegenerative diseases (NDs) represent a group of different neurological disorders, in the majority resulting from genetic and/or environmental factors, having an enormous impact in human health. Alzheimer's disease (AD) is the most prevalent type of ND and dementia. Alzheimer's disease (AD) is a multi-factorial disease deeply associated with impaired cholinergic transmission and oxidative stress (OS), a process that is related with a failure in the antioxidant protective system and/or an increment in reactive species production/accumulation.

Accordingly, neuroprotective agents with an extended therapeutic window, namely those able to restore cholinergic transmission and prevent and/or ameliorate the OS process, are urgently needed. Therefore, the aim of this project has been focused on the design and synthesis of innovative multi-target lipophilic hybrid mitochondriotropic antioxidants using benzoic acid as a scaffold.

In order to achieve this goal, structural changes were performed in natural phenolic antioxidants present in human diet (protocatechuic and gallic acids) by inserting an aliphatic carbon chain spacer linked to a triphenylphosphonium cation (TPP^+). After synthesis, purification and structural identification the *in vitro* antioxidant profile was evaluated using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays. In addition, they have also been screened toward cholinesterase enzymes (AChE and BChE) as they are a key AD targets and molecular docking studies were also performed. Their cytotoxic and neuroprotective profile was evaluated in human neuroblastoma (SH-SY5Y) and in human hepatocellular carcinoma (HepG2) cell lines. The results obtained so far will be presented in this communication.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145-FEDER-006980, PTDC/DTP-FTO/2433/2014 and NORTE-01-0145-FEDER-000028). C. Oliveira, F. Cagide, J. Teixeira, R. Amorim and T. Silva grants were also supported by FCT and FEDER/COMPETE funds.

Poster communication 33

Design and development of novel ammonium cations-functionalized PLGA nanocarriers for targeted drug delivery to central nervous system

Miguel Pinto[a], Carlos Fernandes[a], Fernanda Borges[a]

[a] CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007, Porto, Portugal.

pintomiguelua@gmail.com

Neurodegenerative diseases (NDs), such as Parkinson's (PD) and Alzheimer's (AD) diseases, are among the leading causes of disability and death in the developed world. NDs are multifactorial in nature and slowly progressive bring about huge difficulties in the success of drug discovery and development programs. Despite the growth interest in finding new NDs therapeutic solutions, the most of promising drug candidates developed so far failed in clinical trials as they cannot reach the central nervous system (CNS) in therapeutically relevant doses.[1]

Recent advances in nanomedicine area have provided promising solutions for surpassing the point out constrains, in particular the use of polymeric nanoparticles for the selective transport and delivery of drugs across the blood-brain barrier (BBB).[2] Recent studies have demonstrated that the functionalization of nanocarriers surface with choline derivatives or analogs (i.e. cationic ammonium salts) led to a significant improvement in drug BBB permeability and cellular uptake.[3] Within this framework the use of quaternary ammonium salts grafted polymeric nanoparticles (Figure 1) as drug delivery systems seems to be of great interest to improve drug brain targeting and effectiveness.

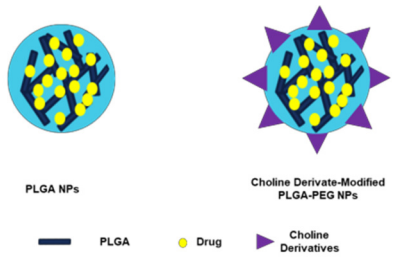

Figure 1: Schematic illustration of choline-analogs surface grafted nanoparticles.

Therefore, the aim of our project was the conjugation of PLGA copolymer with several amonium compounds, synthesis of the nanoparticles and physicochemical characterisation of this type of novel carriers. The results obtained so far will be presented in this communication.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145- FEDER-006980, PTDC/DTP-FTO/2433/2014 and NORTE-01-0145-FEDER-000028). M. Pinto and C. Fernandes were supported by NORTE 2020 funds.

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Poster communication 34

Organization of the local database of the compounds in the academic environment

Črtomir Podlipnik,[a] Marko Jukič[b]

[a] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana*

[b] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana*

crtomir.podlipnik@fkkt.uni-lj.si

The chemistry related research groups at the Universities are usually less focused on the target based synthesis than what is observed in the industry. The research work in academic institutions leads to various synthetics, semisynthetic and natural products, their intermediates and metabolites, with a high degree of diversity. As such, this body of chemical entities could of interest for other academic research groups and the industry endeavors. Knowing this fact, the research groups from the Faculty of pharmacy (FFA) and Faculty of Chemistry and Chemical Technology (FCCT), initiated to develop a system jointly for catalogization, maintaining and storing "research chemicals" in the academic environment and also for small ventures. With integrated chemoinformatics tools this solution will also serve as a platform for further exploration of the chemical space.

Our intranet/internet catalogue is based on standard HTML5 code and interfacing to the backgound databases and server through the use of python. The application is ultralight and may run on any server. The chemicals are physically stored in the locations of both faculties using a modular storage system which enables controlled storage conditions and fast delivery of the compounds (e.g. for screening research scenario). Key approach to our compound management is a unique labelling system of compounds and chemicals. Currently, we use QR codes for the identification of the chemicals stored in vials and the storage racks themselves. The main advantage of the QR codes is that codes are printable on almost every printer and also ubiquitously readable, nowadays even with a common smartphone.

In future, we plan to upgrade our system with an *in house* developed robot based on Arduino platform – such future system would allow screening of the "research" compounds to various targets interesting for industrial applications.

In the end, we would like to find a solution how to integrate our database to Chemotheca, transparently . Therefore we also intend to collect and interface the chemical library to the corresponding intellectual property data, which would enable transparent dissemination and application of our newly constructed library.

Poster communication 35

Funnel-Metadynamics Automated Protocol (FMAP): three steps to disclose drug pharmacodynamics

Stefano Raniolo,[a] Vittorio Limongelli[b]

[a] *Faculty of Informatics, Institute of Computational Science, Università della Svizzera italiana (USI), Lugano, Switzerland*

[b] *Faculty of Biomedical Sciences, Institute of Computational Science, Università della Svizzera italiana (USI), Lugano, Switzerland*

stefano.raniolo@usi.ch

Predicting the thermodynamic properties of the binding process of a drug to its molecular target is of primary relevance to shed light on its mechanism of action and develop new medications. In 2013 our group developed Funnel-Metadynamics (FM).[1] Using FM the ligand binding mode and the accurate estimate of the absolute protein-ligand binding free energy are provided within an affordable computer time. From its development FM has been successfully used by different groups to study ligand/protein and ligand/DNA binding complexes, identifying crystallographic binding modes and predicting experimental binding free energies.[1-5]

The rapid diffusion of FM and the feedback from the users prompted us to develop the Funnel-Metadynamics Automated Protocol (FMAP) that is presented in this talk. FMAP allows disclosing in three steps the whole pharmacodynamics process of a drug to its molecular target, from its unbound state to its final binding mode. FMAP makes use of a graphical user interface (GUI) that allows the interactive preparation of the input files for the FM simulation and the interactive analysis of the results. The GUI guides even inexpert investigators through a step-by-step procedure that is composed of 3 phases: pre-processing, simulation, and post-processing. The final outcome of FMAP is the identification of the ligand binding mode, the metastable states found during the ligand binding mechanism and the accurate estimate of the ligand binding free energy.

In conclusion, FMAP is an accurate, flexible and user-friendly protocol that is expected to impact computer-aided drug design studies in the near future.

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Poster communication 36

(*S*)-Blebbistatin and its derivatives: pharmacological tools for studying myosin II-related diseases

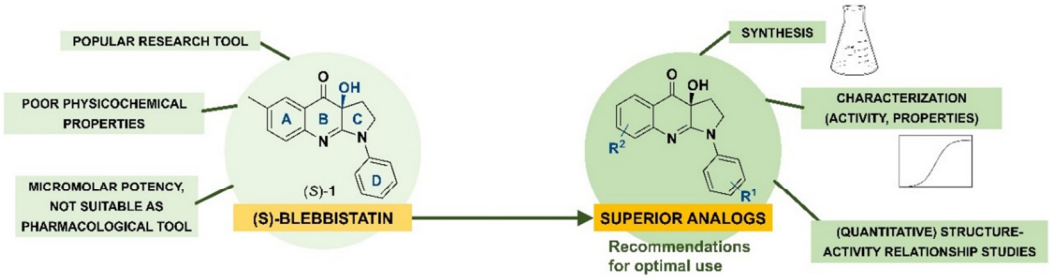
Bart Roman[a,b]

[a] *SynBioC Research Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Coupure Links 653, 9000 Gent, Belgium.*

[b] *Cancer Research Institute Ghent (CRIG), Corneel Heymanslaan 10, 9000 Gent, Belgium*

Email: bart1.roman@ugent.be.

(*S*)-Blebbistatin (*S*)-**1**, a chiral tetrahydropyrroloquinolinone, is a widely used and well-characterized ATPase inhibitor selective for myosin II.[1] The central role of myosin II in many normal and aberrant biological processes has been revealed with the aid of this small molecule. Unfortunately, (*S*)-blebbistatin has severe physicochemical deficiencies that trouble its use in advanced biological systems: low solubility, fluorescence interference, (photo)toxicity and stability issues. We[2,3] have developed a toolbox of (*S*)-blebbistatin analogs in which particular shortcomings have been addressed. This talk will provide a user's guide[1] for their optimal application. Given the multiple roles of myosin II in a diverse range of motility-based diseases, potent and drugable inhibitors of particular isoforms of this protein could be valuable pharmacological tools. The potency of (*S*)-blebbistatin is too low to serve this goal. We and others have strived for potency enhancement via modification of rings A, C and D of the molecule.[1,4,5] We have also analyzed the resulting structure-activity relationships using *in silico* methods.[6]



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Poster communication 37

Cashew nut shell liquid (CNSL) derivatives as sustainable multi-target drugs for the treatment for Alzheimer disease

Michele Rossi,[a] Luciana de Camargo Nascente,[b] Luiz Antonio Soares Romeiro,[b] Maria Laura Bolognesi.[a]

[a] Department of Pharmacy & Biotechnology, Alma Mater Studiorum, University of Bologna, Via Belmeloro 6 40126 Bologna, Italy

[b] Department of Pharmacy, Health Sciences Faculty, University of Brasília, Campus Universitàrio Darcy Ribeiro, 70910-900, Brasília, Brazil

michele.rossi28@unibo.it

The lack of effective treatments for neurodegenerative diseases, as well as a big improvement in longevity are the reasons of an ever-increasing number of people affected by Alzheimer disease (AD) worldwide, particularly in the developing countries. Importantly, considering the high cost of currently available medications, the possibility to develop globally accessible new drugs based on inexpensive resources has gained increasing attention.[1]

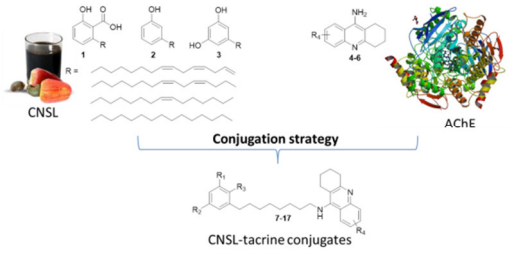

Figure 1: Conjugation strategy to obtain CNLS-tacrine conjugates.

Brazil is one of the main producers of cashew nuts. During the cashew nut processing, an enormous amount of a dark viscous fluid, called cashew nut shell liquid (CNSL), is obtained as a waste material.[1] Long-chain phenolic compounds (**1**-**3**) contained in the inexpensive CNSL show innate multi-target mechanisms of action, including anti-inflammatory and anti-oxidant activity, becoming innovative molecules with potential applications for the treatment of neurodegenerative diseases.[2] In light of this, the aim of this work is the design and synthesis of accessible and sustainable multi-target compounds obtained by combining CNSL derivatives with the well know acetylcholinesterase inhibitor drug, tacrine (**4**). The conjugation strategy (Figure 1) allowed us to obtain innovative CNSL-tacrine conjugates (**7**-**17**) with potential acetylcholinesterase inhibition, anti-inflammatory and, anti-oxidant profile.

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Poster communication 38

VIRTUAL SCREENING FOR INHIBITORS OF THE BmrA PUMP

Duygu Şenturk,[a] Onur Sercinoglu,[a] Pemra Ozbek Sarica,[a] Cedric Orelle,[b] Jean-Michel Jault,[b] and Berna Sariyar Akbulut[a]

[a] *Dept of Bionegineering, Marmara University, Kadikoy, 34722, Istanbul, Turkey*

[b] *Univ Lyon, CNRS, UMR5086 "Molecular Microbiology and Structural Biochemistry", 7 passage du Vercors, 69367, Lyon Cedex 7, France*

berna.akbulut@marmara.edu.tr

Drug efflux mediated antimicrobial antibiotic resistance constitutes a worldwide health problem. The necessity to restore antibacterial susceptibility and to return existing antibiotics into the clinic have made the inhibition of these efflux pumps the center of attention.[1] The current work focuses on the inhibition of the ATP binding cassette drug efflux pump BmrA of *Bacillus subtilus*, which is a homodimer of 575 residues in each chain.[2] It shares a high similarity to the eukaryotic pump P-glycoprotein (P-gp). The current inhibitors of P-gp target two major sites, the nucleotide binding domain (NBD) and the groove between the two halves of the protein that constitute the pump. Taking this as the starting point, MuTaLig chemical library was screened for the best binders that target the NBD site of BmrA. The initial work involved the homology modeling of the BmrA pump protein using Modeller 9.19, taking the *Escherichia coli* pump EcMsbA with full coordinates (the coordinates were kindly provided by Prof. Geoffrey Chang) as the template and *Staphylococcus aureus* SAV1866 (pdb code: 2HYD) for the secondary structure information. The mentioned proteins are bacterial homologs of BmrA. The obtained model was assessed with ProCheck, ProSa and Verify3D for suitability. Virtual screening of the library was performed using an exhaustiveness of 20 with AutoDock Vina 1.1.2[3]. Based on the simulations binding energies were estimated. Our preliminary results have shown that the top scores for the first NBD were obtained with compounds CM28, CM48, CM62, CM67, CM237, CM545, CM552, CM610, CM612, CM616 while the top scores for the second NBD were obtained with CM7, CM22, CM40, CM80, CM85, CM103, CM105, CM149, CM150, CM229. CM237 has a reasonably high score for both NBDs. These results require further experimental verification. This work was supported by Marmara University, Scientific Research Projects Committee (FEN-B-129917-0534).

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Poster communication 39

Neuroprotective Evaluation of Some Phenylacetamide Derivatives Bearing 1,2,4-triazole with Metal Binding Property

Merve Saylam,[a] Sinem Yılmaz,[b,c] Sülünay Parlar,[a] Ayse H Tarikogullari[a]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, Izmir, Turkey*

[b] *Department of Biotechnology, Institute of Science, Ege University, Izmir, Turkey*

[c] *Department of Bioengineering, Faculty of Engineering, University of Alanya Aladdin Keykubat, Antalya, Turkey*

saylammerve@gmail.com

Neurodegenerative diseases such as Alzheimer (AD) and Parkinson (PD) effect millions of people all around the world every year. Although each disease has its own pathology, AD and PD share some similar multifactorial molecular mechanisms such as biochemical abnormalies, aggregation of amyloid-β (Aβ) proteins and neuronal degeneration that results in cell loss[1,2]. It is also known that cholinergic system is affected in both PD and AD. Some of the cholinesterase (ChE) drugs that were approved by FDA such as rivastigmine and donepezil have been tested for PD induced dementia and have been found to improve cognition. Also it has been found that biperiden, which is an anti-parkinson drug in use, inhibited acetylcholine esterase (AChE) enzyme[3,4].

Even though, brain contains high concentration of biometals such as zinc (Zn), copper (Cu) and iron (Fe) physiologically, these metals have also been found in Aβ plaques which is thought to have an important role in the pathogenesis of AD and PD. These biometals are suggested to have roles in pathophysiology through two main pathways: aggregation of Aβ peptide and production of reactive oxygen species induced by Aβ plaques. Therefore, compounds with metal binding ability might be adventageous against neurogenerative diseases [1,5].

According to this literature survey, we have planned to test the metal binding ability and neuroprotectivity of our compounds with *N-substituted-2-(1H-1,2,4-triazole-1-yl)acetamide* general structure that have been reported for their ChE inhibition in our previous study[6]. Metal binding ability was investigated with Zn^{+2}, Cu^{+2} and Fe^{+2} and neuroprotective effect of the title compounds was examined in SH-SY5Y cell lines. The results revealed that all of the compounds have metal binding ability and two of them possessed neuroprotective property.

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Poster communication 40

TACRINE-CANNABINOID 2 RECEPTOR AGONIST HYBRIDS WITH PRONOUNCED *IN VITRO* AND *IN VIVO* ACTIVITIES

Matthias Scheiner[a], Dominik Dolles[a], Sandra Gunesch[a], Massimo Nabissi[b], Jörg Heilmann[c], Tangui Maurice[d] and Michael Decker*[a]

[a] *Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Julius-Maximilian-University of Würzburg, Am Hubland, D-97074 Würzburg, Germany*

[b] *Experimental Medicine and Public Health, University of Camerino, Cia Madonna delle Carceri 9, I-62032, Italy*

[c] *Pharmaceutical Biology Institute of Pharmacy, University of Regensburg, Universitätsstraße 31, D-93040 Regensburg, Germany*

[d] *INSERM UMR-S1198, University of Montpellier, EPHE, F-34095, Montpellier, France*

michael.decker@uni-wuerzburg.de

The current treatment of Alzheimer's disease (AD) is limited to only four approved drugs acting symptomatically. Three drugs (donepezil, rivastigmine and galantamine) share the same mechanism of action by inhibiting acetylcholinesterase (AChE). However, these drugs are only efficient at the early stage of the disease.[1] Due to the multifactorial nature of AD, applying the multitarget approach is well established. Since human butyrylcholinesterase (*h*BChE)[2] and the human cannabinoid 2 receptor (hCB$_2$R)[3] have been established as potential targets[4] for the treatment of later stages of the disease, we herein designed, synthesized and biologically characterized in detail a novel library of hybrid molecules. The hybrids were obtained by coupling a selective hCB$_2$R agonist with the unselective cholinesterase inhibitor tacrine. *In vitro* assays showed ChE inhibition in the one-digit nanomolar range with higher inhibitory activity at *h*BChE while possessing affinity to the hCB$_2$R in the one-digit micromolar range and still acting as receptor agonists. The most promising compounds *in vitro* were tested *in vivo* in an AD mice model,[model described in 4] and showed excellent results already at remarkably low dosage (0.5 mg/kg i.p.) regarding spatial short-term and long-term memory. Tacrine's clinically described dose-dependent hepatotoxicity prompted us to investigate the compounds' effect on liver toxicity in cells and *in vivo*. The compounds are still hepatotoxic, but it could be counteracted by special chemical features of the compounds like a disulfide-bond as well as the low dosage needed for *in vivo* pro-cognitive effects.

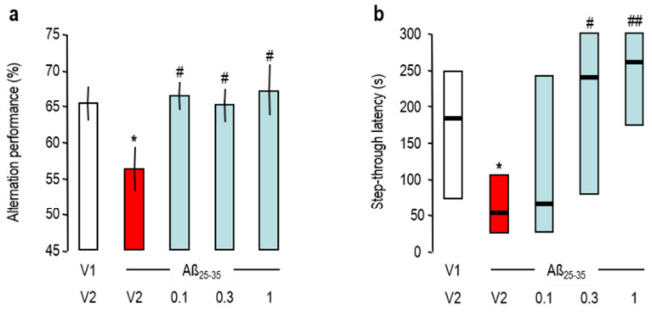

Figure 1 : Effect of a hybrid molecule, administered IP, on Aß$_{25-35}$-induced learning impairments in mice: (a) spontaneous alternation perfomance and (b) step-through latency in the passive avoidance test.

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Poster communication 41

Development of Dual Acting COMT Inhibitors and Iron Chelators: Studies on Synthetic Routes Optimization

Lisa Sequeira[a], Tiago Silva[a], Fernanda Borges[a]

[a]*CIQUP– Chemistry Research Centre of the University of Porto, Porto, Portugal*

ana.sequeira@fc.up.pt

Dopamine (DA) deficiency-neurological disorders such as Parkinson disease (PD) are usually symptomatically treated with the biosynthetic dopamine precursor levodopa that is extensively metabolized by catechol *O*-methyltransferase (COMT).[1] Nitrocatechols are second generation inhibitors of COMT and act as co-adjuvant drugs.[2] Tolcapone, a potent, blood-brain barrier (BBB) permeable and the only centrally-active COMT inhibitor, is associated with a severe risk of hepatotoxicity and its use is very restricted. Commercially available alternatives entacapone and opicapone are safer but act only peripherally.[1] Albeit the nitrocatechol pharmacophore yields potent tight-binding COMT inhibitors, it raises toxicological concerns, particularly for compounds with increased lipophilicity. This makes it particularly difficult to develop BBB-permeable nitrocatechol COMT inhibitors. Non-nitrocatechol COMT inhibitors such as heterocycle catechol mimics (HetCAMs) may thus provide a safer alternative. Moreover, as brain iron accumulation has been acknowledged to contribute to DA depletion this type of compounds can also operate as iron chelators resulting in a disease-modifying outcome.

Accordingly, our project is focused in the development of centrally active dual target drug candidates able to remove accumulated iron in the brain and inhibit COMT, while mitigating the toxicological risks of currently available tolcapone. To achieve the goal two HetCAMs, N-substituted and NH- or O-containing HetCAMs substituted at the C2 position, libraries are being synthesized from a naturally-occurring, cheap and commercially available starting material: kojic acid (**Fig. 1**). The synthetic strategy was designed and optimized. The results obtained so far will be presented in this communication.

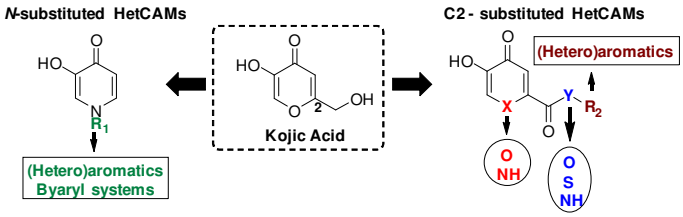

Fig. 1: Drug design strategy followed for the development of HetCAMs.

Acknowledgements: This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980, NORTE-01-0145-FEDER-000028, and POCI-01-0145-FEDER-029164). L. Sequeira and T. Silva grants were also supported by FCT, FEDER/COMPETE and NORTE-2020 funds.

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Poster communication 42

New fluorescent PAMAM dendron with sensor and microbiological activity

Desislava Staneva[a], Evgenia Vasileva-Tonkova[b], Ivo Grabchev[c]

[a]*University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria*
[b]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[c]*Sofia University "St. Kliment Ohridski", Faculty of Medicine, 1407 Sofia, Bulgaria*

grabcheva@mail.bg

A new dendron containing Eosin Y (ED) as a fluorescence unit has been synthesized and characterized. The basic photophysical characteristics of the dendron have been investigated in organic solvents of different polarity and have been compared to the modified monomeric Eosin Y. In *N,N*-dimethylformamide solution, in the presence of metal cations (Li^+, Na^+, K^+, Ag^+, Co^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+} and Cr^{3+}) the newly synthesized dendron quenches its fluorescent intensity depending on the nature of metal cations. The rank of the response can be presented as follows: $Cu^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+} \approx Ag^+ \approx Fe^{3+} \approx Cr^{3+} > Mn^{2+} > Li^+ > Na^+ \approx K^+$. It has also been observed that the effect of pH on the absorption and on fluorescence intensity of dendron ED is less pronounced than that on Eosin Y.

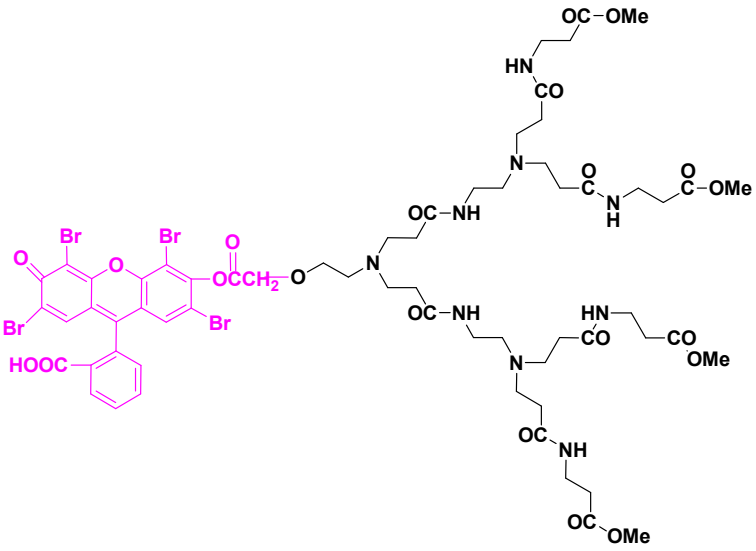

Fig. 1. Chemical structure of PAMAM dendron (ED)

The antimicrobial activity of the new dendron, at a 50 µg/ml concentration was tested in meat-peptone broth towards some model Gram-positive and Gram-negative bacteria and yeasts. ED inhibited the growth of *B. subtilis* and *P. aeruginosa* by 44% and 28%, respectively, what makes this new PAMAM dendron interesting for biological applications.

Acknowledgments: *The authors acknowledge Grant № COST, 01/3-2017, Fund "Scientific Research", Ministry of Education and Science of Bulgaria.*

Poster communication 43

Metformin inhibition of survival signaling in human leukemic cells

[a]Lejla Halilovic, [a]Una Glamoclija, [b]Eugenio Gaudio, [a]Mirza Suljagic

[a]*Molecular Biomedicine, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

[b]*Institute of Oncology Research, Bellinzona, Switzerland*

msuljagic@ius.edu.ba

Numerous studies have shown that metformin decreases cancer risk in diabetic patients (1) and in various clinical trials when addministrated together with first-in-line terapeutic options. This positioned metformin as an interesting repurposing drug and future explorations of signalling mechanisms underlying biguanide–protein interactions are warrented. To evaluate capability of metformin to multi-target key molecules in survival pathways (including NFkB, Mek-ERK and PI3K/Akt/mTOR signaling), we treated diffuse-large B cell lymphoma (DLBCL) cell lines with metformin alone or in combination with selected inhibitors and novel anti-lymphoma agents. For selected inhibitory compounds that are currently in clinical trials, including four anti-lymphoma drugs (BTK inhibitor ibrutinib, the HDAC inhibitor vorinostat, the PI3K delta inhibitor idelalisib, the Bromodomain BRD2/3/4 inhibitor OTX015) we have previously determined IC50 concentrations in four different lymhoma cell lines.

Western blotting was used for evaluating the activity of target kinases in survival signaling cascades after the treatment and co-treatment. We found that metformin exhibited activity in all tested DLBCL cell lines and have shown additive or synergic inhibitory effects even at IC10 combinations.

We have shown that Metformin alone or in co-treatment successfully inhibited the activity of Akt and NFkB signaling together with their downstream substrates. Moreover, when autophagy regulatory proteins were assessed (Fig. 1) inhibitory effect of metformin was seen on p62/SQSTM1, a multi-domain protein adapter, able to bind ubiquitinated proteins and lead them to autophagosomes for degradation.

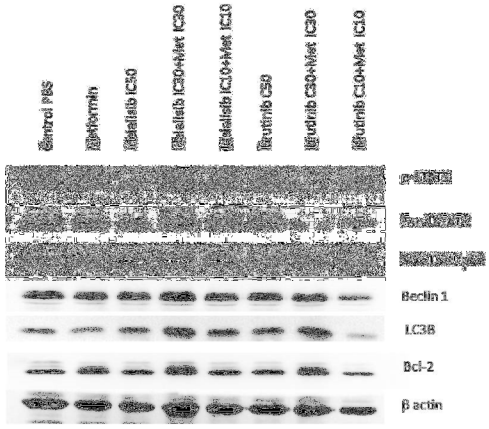

Figure 1. Status of autophagy regulatory proteins in HBL-1 cell line

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Poster communication 44

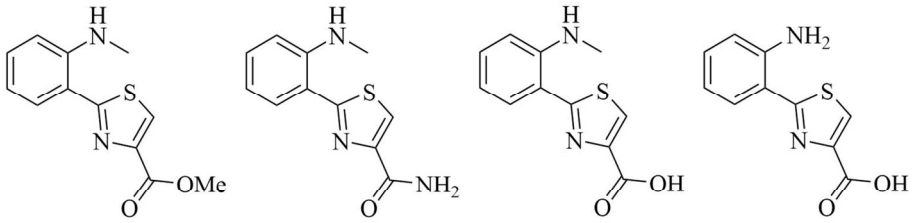
Synthesis of Anithiactin A-C, Thiasporine A, and Synthetic Efforts Towards Enhancing their Biological Activity

Vebjørn Eikemo, I. Caroline Vaaland, Fatemeh Khodakaram, Emil Lindbäck, and Magne O. Sydnes

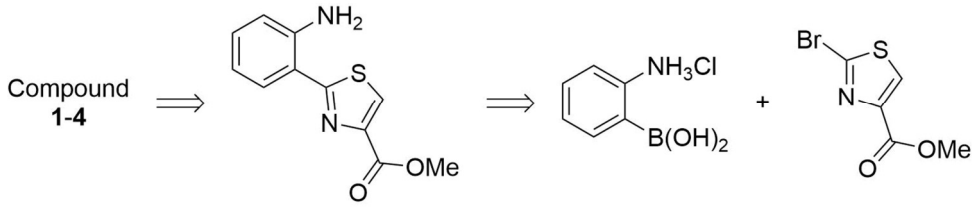
Faculty of Science and Technology, Department of Chemistry, Bioscience, and Environmental Technology, University of Stavanger, NO-4036 Stavanger, Norway

magne.o.sydnes@uis.no

Marine actinobacteria are an important source of naturally occurring biologically active substances, such as anithiactin A-C (**1**-**3**) and thiasporine A (**4**). They were isolated from Streptomyces sp. found in mudflat sediments off the coast of the Korean peninsula[1] and Actinomycetospora sp. native to a mangrove swamp in Vava'u, Tonga.[2,3] Anithiactin A-C displayed moderate acetylcholinesterase inhibitory activity[1,4] and thiasporine A showed cytotoxicity against the non-small-cell lung cancer cell line H2122.[2]



A total synthesis of the four natural products commence with a Suzuki-Miyaura cross coupling between methyl 2-bromothiazole-4-carboxylate and 2-aminophenylboronic acid. Methylation of the amine and hydrolysis and/or aminolysis of the ester group yielded compound 1-4. Additionally, analogues with different substituents on the amine were synthesised with the long-term aim to further enhance their biological activity.



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Poster communication 45

Therapeutic effects of novel methyl jasmonate derivatives on brain cancer cell lines.

Damla Uludag[1], Bilgesu Sucu[1], Ozgecan Savlug[1] , Busra Emine Yazici[1], Mustafa Guzel[1,2], Nihal Karakas[1,3].

[1]İstanbul Medipol University, Regenerative and Restorative Medicine Research Center (REMER), Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810

[2]Istanbul Medipol University, International School of Medicine, Department of Biochemistry, Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810

[3]Istanbul Medipol University, School of Medicine, Department of Medical Biology, Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810

uludagg.d@gmail.com

Glioblastoma multiforme (GBM) is the most common and malignant form of brain tumors in adults. Invasive GBM cells rapidly proliferate and spread into nearby tissues . Patients can only survive for an average of 12 months after diagnosis. Standard therapies for GBM includes surgical resection with adjuvant radiotherapy and chemotherapy. Despite extensive treatment, disease progression is associated with adverse clinical outcomes. In many drug therapies for GBM, there are no effective results due to blood-brain barrier permeability, insufficient therapeutic effects *in vivo* and/or halh life of molecules.

Targeting increased glycolysis in cancer cells is a novel and promising method for cancer treatment. Glycolysis inhibition can be made without significant side effects, and such treatment will contribute to the majority of known cancer therapies. Under the Warburg effect, cancer cells produce energy with high glucose consumption and then lactate fermentation in the cytoplasm, even in the presence of oxygen. This effect suports proliferation, increased invasive ability, and apoptosis resistance in cancer cells [1]. Hexokinase-2 (HK-2) plays an important role in the development of the Warburg phenotype. This enzyme is a necessary rate limiting step for both glycolysis and ultimately oxidative phosphorylation [2]. Thereby, HK-2 has a critical role in cancer progression, and its overexpression is associated with poor prognosis in many types of cancers. Previously, upregulation and overexpression of HK-2 in GBM cells have been shown to play a key role in regulating metabolic pathways [3]. Therapeutic strategies modulating the Warburg effect, such as HK-2 targeting, are thought to affect GBM cells growth and therapeutic sensitivity. In this context, inhibition of HK-2 enzyme is expected to prevent the GBM cells from being fed and inhibited tumor growth in these cells. Although jasmonates have been reported to inhibit proliferation and induce apoptosis in various cancer cells, anti-cancer effects of Methyl Jasmonate (MeJa) on GBM cells have been poorly investigated while there is no reports on therapeutic response of GBM cells to MeJa derivatives .

In this study, we investigated anti-cancer effects of novel methyl jasmonate analogues produced by our research group as compared to MeJa on GBM cell lines which have different levels of resistancy to current therapeutics in clinics.

This COST project (215S890) is funded by TUBITAK.

Keywords: Cancer Methabolism, Glioblastoma Multiforme, Methyl Jasmonate, Hexokinase inhibitors.

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Poster communication 46

Multifunctional ligands targeting cholinesterases, 5-HT$_6$ receptors and β-amyloid.

Anna Wieckowska,[a] Barbara Malawska,[a]

[a]*Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Str. 30-688 Kraków, Poland*

anna.wieckowska@uj.edu.pl

Alzheimer's disease (AD) has a mounting impact on the society as we observe a constant increase in its incidence and lack of effective therapy. AD is a complex neurodegenerative disease caused by a multitude of factors among which aggregation and accumulation of amyloid-β (Aβ) deposits and severe changes in neurotransmitters' levels in brain play major role. Currently available drugs developed according to the '*one-disease-one-target*' paradigm are not sufficient to cure the disease therefore we used multi-target-directed ligands (MTDLs) strategy.[1]

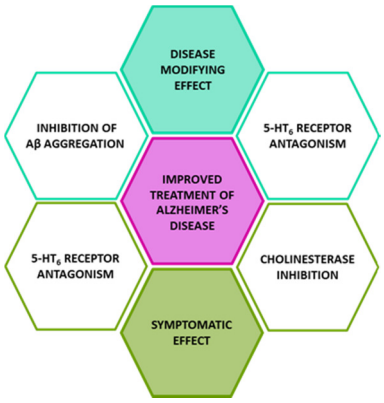

Figure 1: Multi-target-directed ligands combining symptomatic and disease-modifying effects.

We based our approach on the results of clinical trials where 5-HT$_6$ antagonists improved cognitive functions in donepezil-treated patients with moderate AD. In the presented MTDLs we have combined pharmacophore fragments that provide them with cholinergic effect caused by inhibition of cholinesterases and antagonism against 5-HT$_6$ receptors. The compounds display also anti-aggregation properties against Aβ. Due to their mechanism of action they have potential to improve cognitive functions (cholinesterase inhibition, 5-HT$_6$ antagonism), alleviate psychological symptoms accompanying AD (5-HT$_6$ antagonism), and affect causative processes (Aβ inhibition).[2,3]

Acknowledgments: this work was supported by the National Science Centre, Poland, grant number 2016/23/D/NZ7/01328 and 2016/21/B/NZ7/01744.

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Poster communication 47

MINING STRUCTURAL DATA FROM THE PROTEIN DATA BANK

Loic Dreano, Ashenafi Legehar, Evgeni Grazhdankin, Alexandre Borrel, Henri Xhaard[a]

[a] *Computational Drug Discovery group, Faculty of pharmacy, P.O. BOX 56, FI-00014 University of Helsinki, Finland*

henri.xhaard@helsinki.fi

The Protein Data Bank (PDB) holds around 130,000 protein structures that provides a wealth of information about molecular interactions and by extension ligand binding promiscuity, the central theme of the MuTaLig action. Filtering the complexes of interest is usually conducted at different levels in order to build datasets where for example the resolution, protein chain and ligand redundancies, interactions with metals, or simply associated features have been controlled. Furthermore, the queries such as molecular distances extracted require often to be modified many times during the course of a project. This is usually done using collections of scripts organized into workflows. Examples of organizing the data into databases are rare, yet they represent a powerful and time-efficient ways to conduct data mining studies.

In this communication, I will present our efforts to extract data from the PDB[1,2], in particular to mine for structural isosteres of phosphate groups with surprising results[2]. I will then follow to present current unpublished work in the laboratory. We have build a relational database, developed in PostgreSQL to mine molecular interactions in the PDB, as well as associate key features. Applications under development in particular to knowledge-based scoring at the atomistic level will be presented.

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Poster communication 48

Targeting Different Types of Cancers by Novel Methyl Jasmonate Derivatives

Busra Emine Yazici[1], Bilgesu Onur Sucu[1], Ozgecan Savlug[1] , Sukran Ozdatlı[1], Damla Uludag[1], Nihal Karakas[1,2], and Mustafa Guzel[1,3]

[1]*İstanbul Medipol University, Regenerative and Restorative Medicine Research Center (REMER), Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810,Turkey*

[2] *Istanbul Medipol University, School of Medicine, Department of Medical Biology,KavacikCampus, Kavacik-Beykoz/ISTANBUL 34810, Turkey*

[3]*Istanbul Medipol University, International School of Medicine, Department of Medical Pharmacology, Kavacik Campus, Kavacik-Beykoz/ISTANBUL 34810, Turkey*

beyazici@st.medipol.edu.tr

Cancer cells tend to have accelerated glucose metobolism and develop several metabolic alterations to survive in unfavorable microenviroments. One of the difference in metabolism between tumor cells and non-tumourigenicnormal cells is thattumor cells prefer aerobic glycolysis rather than oxidative phosphorylation even there is oxygen in the enviroment which is termed as Warburg Effect [(1)].

Hexokinases catalyse first step of glucose metabolism by phosphorilating glucose to glucose-6-phosphate. Hexokinase 2 (HK2) is a subtype of hexokinase enzymes, andas a metabolic target in cancer therapy, HK-2 is highly expressed in cancer cells than in normal cells. HK2 can bind to the mitochondrial VDAC (Voltage-dependent anion channel) [(2)] and VDAC is also overexpressed in cancer cells[(5)]. One of the anti-cancer strategy that targets energy metabolism of the cell is use of HK2 inhibitors to stop tumour growth.

Jasmonates, a family of plant stress hormones, act through hexokinases and show anti-cancer activity by effecting mitocondria directly. It has been reported that Methyl Jasmonate binds specifically to mammalian hexokinase and disrupts its interaction with VDAC, and causes detachment of hexokinase from the mitochondria followed by cytochrome c release[(4)]. It is also reported that Jasmonic acid and methyl jasmonate are able to inhibit four isoforms of human aldo-keto reductase superfamily (AKR1C) [(3)]. Therefore, Methyl Jasmonate has beenstudied on various cancers as a novel anti-cancer agent with promosing results.

In this study, we designed and synthesized novel Methyl Jasmonate derivatives that has the multiple target potential for HK2 as well AKR1C inhibition and investigated theirbiological activity and anti-cancer effects on cancer cells from different origins. We will highlight those results in our poster and oral presentation.

KeywordsCancer metabolism, hexokinase inhibitors, methyl jasmonate, anti-cancer drugs

Note: This COST project (215S890) is funded by TUBITAK. We kindly appreciate for their support.

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