First WG Meeting

BOOK OF THE ABSTRACTS

COST ACTION CA15135

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

POLY-PHARMACOLOGY EXPANDING PAUL EHRLICH'S MAGIC BULLET CONCEPT

Hotel Novotel Budapest Danube - Budapest, November 19-20 2016

www.mutalig.eu

INTRODUCTION

The MuTaLig COST Action aims to catalyze the interactions among highly-qualified research teams working in the emergent Medicinal Chemistry branch, known as multi-targeting or poly-pharmacology. Last July in Lugano MuTaLig COST Action officially started its scientific activity with a successful 1st annual meeting. As result of that event, some collaborations started within the network and first three STSM mobility programs were activated. On the other hand, there is still the need to consolidate the knowledge of the network and to focus on more specific issues, such as those of the four working groups compelling the COST Action. The topics of the WG meeting will span around medicinal chemistry, from synthetic chemistry, natural products and biophysics to theoretical chemistry, molecular modelling and biological screening. Experts in this field, including those from renowned pharma companies, were invited to give plenary lectures. A large space will be dedicated to short communications, given by MC board components as well as by young investigators, belonging to research institutions located in different places of thirty-one MuTaLig COST Action parties, according to the gender balance. The two days will be completed by flash communication session and a final round table. In conclusion, the 1st Working Group meeting represents another essential occasion of knowledge and exchange information among the research teams.

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

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

Stefano Alcaro

Università “Magna Græcia” di Catanzaro (Italy)

Chair of CA15135 COST Action and LOS of the meeting

alcaro@unicz.it

PROGRAM

Saturday November 19st 2016

8.30 Registration

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

Session I "Experts on multi-targeting drug discovery"
moderator Julio Alvarez-Builla (PE Coordinator)
Alcalá University, Madrid (Spain)

9.15 PL1 **Cariprazine: A short overview of newest pharmaceutical and medical milestone in the psychiatry**
György NÉMETH - Chemical Works of Gedeon Richter Plc., Budapest (Hungary)

10.00 PL2 **Through privileged scaffolds to multi-target drugs: isatin hybrids as anti-viral and anti-tumour agents**
Elias MACCIONI - University of Cagliari (Italy)

10.45 *Coffee break*

Session II "New chemical entities (WG1)"
Moderator Danijel KIKELJ (WG1 leader)
University of Ljubljana (Slovenia)

11.00 SC1 **Multi-target-directed-ligands for Alzheimer's disease**
José MARCO-CONTELLES, CSIC, Madrid (Spain)

11:15 SC2 **Inhibition of multiple targets by novel bisindolylmaleimides**
Florence O. MCCARTHY, University College Cork (Ireland)

11:30 SC3 **Chromone as a promising scaffold for the development of multitarget ligands for neurodegenerative diseases**
Joana REIS, University of Porto (Portugal)

11:45 SC4 **Development of new indole derivatives as multitarget agents for the treatment of neurodegenerative diseases**
Maria Rosa BUEMI, University of Messina (Italy)

12:00 SC5 **Design, synthesis and biological evaluation of novel 1-benzylpyrrolidin-3 amine derivatives as potential inhibitors of beta-secretase and cholinesterases**
Wichur TOMASZ, Jagiellonian University Collegium Medicum, Kraków (Poland)

12:15 SC6 **Navigation in bile acid chemical space: discovery of novel FXR and GPBAR1 modulators**
Claudia FINAMORE, University of Naples "Federico II" (Italy)

12:30 SC7 **Synthesis of triclosan derivatives and their biological activity**
Rudolf VOSÁTKA, Charles University, Hradec Králové (Czech Republic)

12:45 SC8 **Isatin-thiazolidinone hybrids as DNA G-quadruplex selective stabilisers: a new potential approach to multitarget anticancer agents**
Claudia MELIS, University of Cagliari (Italy)

13:00 SC9 **Interaction of ATP-competitive DNA gyrase inhibitors with the cellular chaperone**

HSP90 as a potential pathway to inhibit viral replication
Tihomir TOMAŠIČ, University of Ljubljana (Slovenia)

13:15 SC10 **Rational Design and Synthesis of Novel Amidine-Based Scaffolds as BACE1 Inhibitors**
Myriam CIORDIA JIMÉNEZ, Universidad CEU San Pablo, Madrid (Spain) – *PE awarded*

13.30 *Lunch*

Session III "Experts on computational methods"
Moderator Stefano ALCARO (CA15135 Chair & LOS)
Università "Magna Græcia" di Catanzaro (Italy)

14.30 PL3 **Setting up a web-based molecular structure database with open-source software**
Norbert HAIDER - University of Vienna (Austria)

15.15 PL4 **Computational chemogenomics: Is it more than inductive transfer?**
Dragos HORVATH - CNRS Strasbourg, (France)

16.00 *Coffee break and poster session*

Session IV "Chemical databases (WG3) and computational methods for multiple ligand design and discovery (WG4)"
Moderator Hanoch SENDEROWITZ (WG4 leader)
Bar-Ilan University, Ramat-Gan (Israel)

16:30 SC11 **Let's develop together an exchange virtual compounds computational platform: Updates!**
Carmine TALARICO, University "Magna Græcia" di Catanzaro (Italy)

16:45 SC12 **Natural Compounds from NuBBE database as Inhibitors of Zika Virus – E Protein**
Črtomir PODLIPNIK, University of Ljubljana (Slovenia)

17:00 SC13 **A computational multitarget screening for the in silico identification of bioactive compounds**
Roberta ROCCA, University "Magna Græcia" di Catanzaro (Italy) – *PE awarded*

17:15 SC14 **Validation of Docking and Dynamic protocol for the study of a new class of Oxadiazoles able to inhibit MAO-B and to prevent Neuronal Oxidative damage**
Giulia BIANCO, University of Cagliari (Italy) – *PE awarded*

17:30 Flash communications selected from the poster session

19:00-21:00 **Social Program**

Sunday November 20st 2016

Session V "Biological targets and assessment of biological data (WG2)"
Moderator Eugenio GAUDIO (WG2 leader)
Oncology Research Institute, Bellinzona (Switzerland)

9:00 SC15 **Dual enzyme inhibitors: a promising multi-target approach to cancer therapy**
Rita MELEDDU, University of Cagliari (Italy)

9:15 SC16 **Surface proteomics, a step to simplify the rapid prototyping of nanodrugs**
Susana CRISTOBAL, Linköping University (Sweden)

9:30 SC17 **Screening the central effects of multitarget agent, non-imidazole H3 receptor antagonist/inverse agonists**
W. Agnieszka FOGEL, Medical University of Lodz (Poland)

9:45 SC18 **Targeting metastasis-associated in colon cancer-1 (MACC1) via HGF-Met-MAPK pathway as novel predictive marker for breast cancer**
Daria LER, PZU Moja Klinika-General Hospital Sarajevo (Bosnia and Herzegovina)

10:00 SC19 **Hydroxycinnamic acid as a valid scaffold for the development of CNS multitarget drugs**
Tiago SILVA, University of Porto, (Portugal) – *PE awarded*

10:15 SC20 **Discovery of a natural feruloyl ester as single inhibitor of HIV-1 RT and synthesis of its dual inhibitors analogues**
Vijay SONAR, University of Cagliari (Italy)

10:30 SC21 **Multitargeting prosurvival pathays in human leukemic cells by metformin and thymoquinone**
Mirza SULJAGIC, International University of Sarajevo (Bosnia and Herzegovina)

11:00 SC22 **Targeting dietary antioxidants to mitochondria as a therapeutic solution for mitochondrial oxidative stress related diseases**
José TEIXEIRA, University of Porto (Portugal) – *PE awarded*

11:15 *Coffee break*

Round table "Status of the WG activites of MuTaLig COST Action"
open to all participants - Moderator Fernanda BORGES (CA15135 Vice-Chair)
University of Porto (Portugal)

11:45

12:45 **Closing remarks**

13:00 **Lunch**

Plenary lectures

Plenary Lecture 1

First of class treatment of negative symptoms of schizophrenia

Gyorgy Nemeth

Medical Division of Gedeon Richter Plc. (Chief Medical Officer), Gyomroi ut 19-21., Budapest, Hungary

gy.nemeth@richter.hu

Background:

Cariprazine is an orally active and potent dopamine D3/D2 receptor partial agonist with preferential binding to D3 receptors and partial agonist at serotonin 5-HT1A receptors. Cariprazine is the first and only atypical antipsychotic that demonstrates this balanced dual engagement of the DR_3R and DR_2R receptor systems, which, next to its antipsychotic effect may confer additional benefits such as improvement in negative symptoms and enhanced cognition. Cariprazine therefore was developed for the treatment of schizophrenia and for the treatment of schizophrenia with predominant negative symptoms.

Methods:

Beside 25 phase I clinical trials the cariprazine schizophrenia program included 3 short term studies in acute exacerbations, 1 long-term maintenance of effect study, 1 special clinical study in patients with predominant negative symptoms of schizophrenia and 2 long term safety studies. The primary and secondary efficacy parameters in the short term studies were change from baseline to end in the Positive and Negative Syndrome Scale (PANSS) total score and Clinical Global Impressions – Severity scale (CGI-S) score, respectively compared to placebo. The primary efficacy parameter in the maintenance of effect study was the time to first relapse during the double blind period in comparison with placebo. The primary and secondary efficacy parameters in the predominant negative symptom study were change from baseline to end in the PANSS factor score for negative symptoms (PANSS-FSNS) and in the Personal and Social Performance Scale (PSP) respectively, in comparison with risperidone.

Results:

In each of the 3 short-term studies, significant improvements were seen for cariprazine relative to placebo in the primary and secondary efficacy parameters, using analysis of covariance (ANCOVA) with last observation carried forward (LOCF) and mixed-effects model for repeated measures (MMRM) methods to fill the missing endpoint. Cariprazine demonstrated robust efficacy in maintaining antipsychotic effect: by the end of the double-blind-period 47.5% of placebo-treated patients and 24.8% of cariprazine-treated patients had a relapse of schizophrenia symptoms. Cariprazine demonstrated robust efficacy on predominant negative symptoms of schizophrenia in a dose-range of 3-6 mg, with a target dose of 4.5 mg: There was a statistically significant difference ($P = 0.002$) in favor of cariprazine over risperidone for the primary efficacy parameter PANSS-FSNS and for the secondary efficacy parameter, PSP ($P < 0.001$). The most common adverse events were akathisia, insomnia and headache; however the last two occurred with similar frequency also in the placebo groups. Cariprazine had a favorable safety profile considering prolactin levels, weight gain and hematological parameters.

Discussion:

Overall the efficacy data gained shows that cariprazine improves a broad range of schizophrenic symptoms in all stages of schizophrenia and has a similar safety profile like other marketed antipsychotics. However cariprazine is unique in treating predominant negative symptoms of schizophrenia, a first of class approach in a field where there are no available therapies at the moment and which is considered to be a high unmet medical need, as it represents a burdensome and disabling disease for patients, caregivers and society.

Plenary Lecture 2

Through privileged scaffolds to multi-target drugs: isatin hybrids as anti-viral and anti-tumour agents

Elias Maccioni,[a] Filippo Cottiglia,[a] Stefano Alcaro,[b] Peter Matyus,[c] Rita Meleddu,[a] Giulia Bianco,[a] Antonella Arridu,[a] Simona Distinto

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

[b] *Department of Health,* Magna Graecia University, Campus "S. Venuta", Viale Europa, 88100 Catanzaro, Italy;

[c] *Department of Organic Chemistry, Semmelweis University Hogyes Endre u. 7 Budapest, H-1092, Hungary.*

maccione@unica.it

During the last ten years the drug discovery process has undergone huge changes. It is now clear that the era of combinatorial synthesis, when thousands of compounds were synthesised and tested to pursue hits discovery, has been overtaken by the rational design of small libraries of potentially active compounds. In this respect, the discovery if Imatinib, originated by a rationale, knowledge driven drug discovery process, could be considered as a milestone. At the same time, it is now commonly accepted, within the scientific community, that multifactorial diseases need multi-target drugs.[1] This completely new approach has led to the development of new strategies to face the challenge of the identification of new multi-target directed therapeutic agents. An advantageous approach could be the design of new bioactive molecules starting from a privileged scaffold. The term privileged scaffold was firstly used by Evans [2] to describe a structural subunit shared by several drugs acting towards different biological target. On these basis, the isatin moiety could be considered as a privileged scaffold and might represent a valid starting point for the design of new multi-target agents.[3-5]

References:

1. Anighoro, A., J. Bajorath, and G. Rastelli, *Polypharmacology: Challenges and Opportunities in Drug Discovery.* J. Med. Chem., 2014. **57**(19): p. 7874-7887.

2. Evans, B.E., et al., *Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists.* Journal of Medicinal Chemistry, 1988. **31**(12): p. 2235-2246.

3. Agamennone, M., et al., *Fragment-Based Discovery of 5-Arylisatin-Based Inhibitors of Matrix Metalloproteinases 2 and 13.* ChemMedChem, 2016: p. Ahead of Print.

4. Hou, J., et al., *LJNK, an indoline-2,3-dione-based aminopeptidase N inhibitor with promising antitumor potency.* Anti-Cancer Drugs, 2016. **27**(6): p. 496-507.

5. Rane, R.A., et al., *A Recent Perspective on Discovery and Development of Diverse Therapeutic Agents Inspired from Isatin Alkaloids.* Curr. Top. Med. Chem. (Sharjah, United Arab Emirates), 2016. **16**(11): p. 1262-1289.

Plenary Lecture 3

Setting up a web-based molecular structure database with open-source software

Norbert Haider

Department of Pharmaceutical Chemistry, University of Vienna, Austria

norbert.haider@univie.ac.at

In Medicinal Chemistry nowadays, handling of smaller or larger sets of molecular structures together with associated data (describing the preparation, characterisation, properties, activities, etc., of molecules) is an essential task for all scientists involved in a research project, it is no longer the exclusive domain of IT specialists. For typical operations like storage, retrieval, comparison, analysis, archiving, or exchange of such data records, various commercial and non-commercial software solutions are available. Apart from the financial aspect, open-source software offers the advantage of practically unlimited flexibility with respect to data types and algorithms as well as independence from producers of proprietary closed-source software. In this presentation, the creation of a web-based compound database will be explained, using a combination of widely used general-purpose open-source software (operating system, web server, database engine, scripting language) and a collection of custom-made cheminformatics tools that are open-source, as well. Various aspects regarding the installation, administration, everyday operation and some of the underlying cheminformatics principles will be discussed for the MolDB6 software package.[1,2]

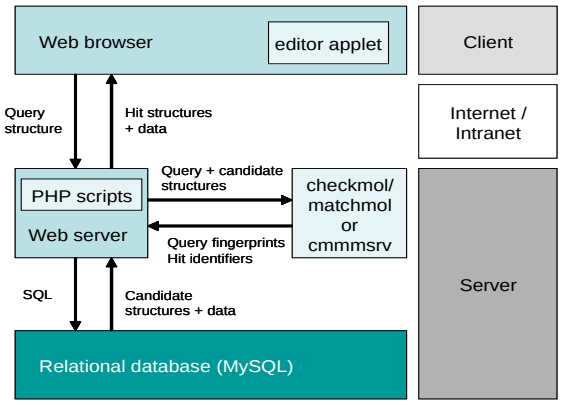


Figure 1: Interaction of the principal software components in a MolDB6 structure database

Besides standard features like text search, structure/substructure/similarity search and reaction search, the system offers an option for functionality pattern searches, making use of an automatic recognition and assignment of the most common, drug-relevant funtional groups (approx. 200) for all stored structures.

References:

[1] MolDB6 homepage, http://merian.pch.univie.ac.at/~nhaider/cheminf/moldb6.html

[2] Haider, N. Functionality Pattern Matching as an Efficient Complementary Structure/Reaction Search Tool: an Open-Source Approach. *Molecules*, **2010**, *15*, 5079-5092.

Plenary Lecture 4

Computational Chemogenomics: is it more than inductive transfer?

J. B. Brown,* Yasushi Okuno,* Gilles Marcou,° Alexandre Varnek,° Dragos Horvath°

** Department of Clinical System Onco-Informatics, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan.*

° Laboratoire de Chemoinformatique, UMR 7140 CNRS, Univ. Strasbourg, 1, rue Blaise Pascal, 67000 Strasbourg, France.

dhorvath@unistra.fr

High-throughput assays challenge us to extract knowledge from multi-ligand, multi-target activity data. In QSAR, weights are statically fitted to each ligand descriptor with respect to a single endpoint or target. However, computational chemogenomics (CG) has demonstrated benefits of learning from entire grids of data at once, rather than building target-specific QSARs.

A possible reason for this is the emergence of inductive knowledge transfer (IT) between targets, providing statistical robustness to the model, with no assumption about the structure of the targets. Relevant protein descriptors in CG might allow to learn how to dynamically adjust ligand attribute weights with respect to protein structure. Hence, models built through explicit learning by including protein information (EL), while benefitting from IT enhancement, should provide additional predictive capability, notably for protein deorphanization.

This interplay between IT and EL in CG modeling is not sufficiently studied. While IT is likely to occur irrespective of the injected target information, it is not clear whether and when boosting due to EL may occur. EL is only possible if protein description is appropriate to the target set under investigation. The key issue here is the searching for evidence of genuine EL exceeding expectations based on pure IT.

We explore the problem in the context of Support Vector Regression, using > 9400 pK_i values of 31 GPCRs, where compound-protein interactions are represented by the concatenation of vectorial descriptions of compounds and proteins. This provides a unified framework to generate both IT-enhanced and potentially EL-enabled models, where the difference is toggled by supplied protein information. For EL-enabled models, protein information includes genuine protein descriptors such as sequence counts.

EL- and IT-based methods were benchmarked alongside classical QSAR, with respect to cross-validation and deorphanization challenges. While EL-enabled strategies outperform classical QSARs and favorably compare to similar published results, they are, in all respects evaluated, *not* strongly distinguished from IT-enhanced models. Moreover, EL-enabled strategies failed to prove superior in deorphanization challenges. These results show that the field of protein descriptor research needs further improvements to truly realize the expected benefit of EL.

Short communications

Short communication 1 - Working Group 1

Multi-Target-Directed-ligands for Alzheimer's disease

José Marco-Contelles

Laboratory of Medicinal Chemistry [Institute of Organic Chemistry (CSIC); 3, Juan de la Cierva, 28006-Madrid (Spain)]

iqoc21@iqog.csic.es

In this communication we will update our recent results on the synthesis and biological evaluation of *N*-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (**ASS234**)[1-5] (Figure 1), our multipotent molecule able to bind ChE and MAO enzymes, based on donepezil, for the potential treatment of Alzheimer's disease.

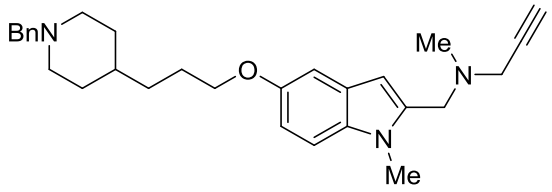


Figure 1: Structure of compound **ASS234**.

References:

[1] Esteban, G.; Allan, J.; Samadi, A.; Mattevi, A.; Unzeta, M.; Marco-Contelles, J.; Binda, C.; Ramsay, R. R. Kinetic and structural analysis of irreversible inhibition of human monoamine oxidases by ASS234, a multi-target compound designed for use in Alzheimer's disease. *BBA Proteins and Proteomics* **2014**, *1844*, 1104-1110.

[2] del Pino, J.; Ramos, E.; Bautista Aguilera, O. M.; Marco-Contelles, J.; Romero, A. Wnt signaling pathway, a potential target for Alzheimer's disease treatment, is activated by a novel multitarget compound ASS234. *CNS Neurosci.& Therapeutics* **2014**, *20*, 568-570.

[3] Stasiak, A.; Mussur, M.; Unzeta, M.; Samadi, A.; Marco-Contelles, J. L.; Fogel, W. A. Effects of novel monoamine oxidases and cholinesterases targeting compounds on brain neurotransmitters and behavior in rat model of vascular dementia. *Curr. Pharmaceutical Design* **2014**, *20*, 161-171.

[4] Bolea, I.; Gella, A.; Monjas, L.; Pérez, C.; Rodríguez-Franco, M. I.; Marco-Contelles, J. L.; Samadi, A.; Unzeta, M. Multipotent, permeable drug ASS234 inhibits Abeta aggregation, possesses antioxidant properties and protects from Abeta-induced apoptosis in vitro. *Curr. Alzheimer Res.* **2013**, *9*, 797-808.

[5] Bolea, I.; Juárez-Jiménez, J.; de los Ríos, C.; Chioua, M.; Pouplana, R.; Luque, F. J.; Unzeta, M.; Marco-Contelles, J.; Samadi, A. Synthesis, biological evaluation, and molecular modeling of donepezil and N-[(5-(benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine hybrids as new multipotent cholinesterase/monoamine oxidase inhibitors for the treatment of Alzheimer's disease. *J. Med. Chem*. **2011**, *54*, 8251-8270.

Short communication 2 - Working Group 1

Inhibition of multiple targets by novel bisindolylmaleimides

Kevin D. O'Shea,[a] Hannah M. Winfield,[a] Michael M. Cahill,[a] Larry T. Pierce,[a] Florence O. McCarthy.[a]

[a] *Department of Chemistry and ABCRF, University College Cork, Western Road, Cork, Ireland*

f.mccarthy@ucc.ie

Since the discovery of broad spectrum kinase inhibition of the indolocarbazole staurosporine (**1**), many analogues have been synthesised to obtain compounds with higher potency and selectivity. A simple modification of staurosporine has led to the bisindolylmaleimides for which multiple modes of biological action are also reported. One such example ruboxistaurin (**2**), a highly selective inhibitor of protein kinase C-β, but many others have reported polypharmacology including the inhibition of multiple protein kinases and other targets in cancer therapy.

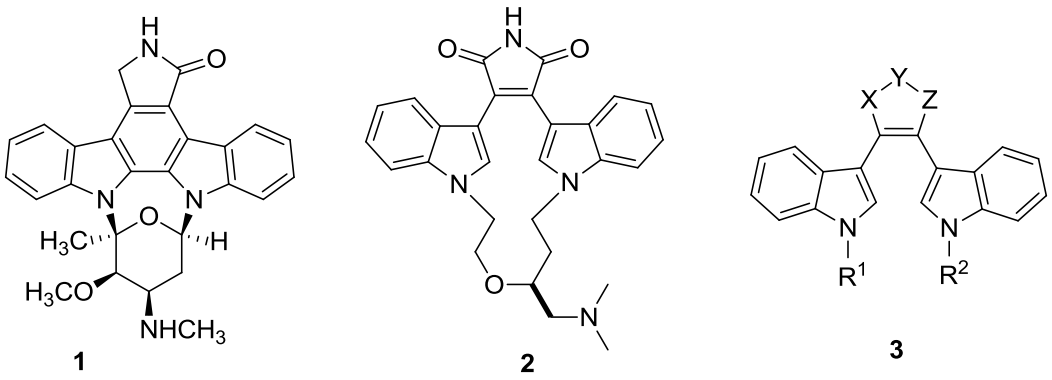


Figure 1: Structures of Staurosporine, ruboxistaurin and a representative bisindolemaleimides.

Our work is focused on the production of highly potent novel anticancer agents through modification of the bisindolylmaleimide structure utilising novel heterocycles on the connecting ring (**3**) and substitution of the indoles. To date we have produced several novel heterocycles (aminopyrazole, imidazole, uracil, thiouracil, pyrazolone, etc.)[1,2] as a replacement for the lactam/maleimide in structures of type (**2**). Biological assessment including topoisomerase I and topoisomerase II inhibition assays and full characterisation via the NCI 60 cell line screen has been completed and significant anticancer activity will be described from individual templates within this compound library.[3]

References:

[1] L. T. Pierce, M. M. Cahill and F. O. McCarthy. Synthesis of novel 3,4-diaryl-5-aminopyrazoles as potential kinase inhibitors. *Tetrahedron* **(2011)** 67 (25):4601-4611.

[2] L. T. Pierce, M. M. Cahill and F. O. McCarthy. Design And Synthesis Of Novel 5,6-Bisindolylpyrimidin-4-Ones Structurally Related To Ruboxistaurin (LY333531). *Tetrahedron* **(2010)** 66 (51):9754-9761.

[3] L. T. Pierce, M. M. Cahill, H. J. Winfield and F. O. McCarthy. Synthesis and Identification of Novel Indolo[2,3-*a*]pyrimido[5,4-*c*]carbazoles as a New Class of Anti-cancer Agents. *Eur. J. Med. Chem.* **(2012)** 56: 292-300.

Short communication 3 - Working Group 1

Chromone as a promising scaffold for the development of multitarget ligands for neurodegenerative diseases

Joana Reis,[a] Fernando Cagide,[a] Eugenio Uriarte,[b] Maria Isabel Rodríguez Franco,[c] 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] *Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago Compostela, Campus Vida, 15782, Santiago de Compostela, Spain;*

[c] *Instituto de Química Médica (CSIC), c/ Juan de la Cierva, 3 28006, Madrid, Espana.*

jocostareis@gmail.com

Neurodegenerative diseases (ND's) are characterized by a progressive dysfunction of neuronal systems, and as a result a deterioration of the cognitive function. These disorders, often associated with atrophy of the affected central or peripheral structures of the nervous system, include Alzheimer's (AD) or Parkinson's (PD). There is now a comprehensive understanding that ND's etiology is multifactorial, with interlinked pathways and similar pathological features. As a result, the development of multitarget-directed-ligands (MTDLs) have been increasingly exploited to develop drugs hitting different druggable targets, namely acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) or monoamine oxidases (MAOs).[1]

Chromone is by now recognized as a privileged structure useful for the design of libraries in diverse drug discovery programs.[2,3] Accordingly, our project aims at the rational discovery of new chromone-based derivatives possessing dual-target activity (MAO-B and AChE). The development of the current library follows a molecular hybridization strategy with the compounds tested so far displaying nanomolar to low microlar activity towards both enzymes. The best candidates display positive blood-brain barrier permeability and no cytotoxic effects have been observed in the preliminary cell viability studies. Molecular modeling and mechanistic enzymatic studies to assess their mechanism of action are also in progress. A general overview of the synthetic strategies pursued in this work, as well as the biological results will be presented in this communication.

References:

[1] Cavalli, A.; Bolognesi, M.L.; Minarini, A.; Rosini, M.; Tumiatti, V; Recanatini, M.; Melchiorre, C.J. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.*, **2008**, *51*, 347-72.

[2] Gaspar, A.; Matos, M. J.; Garrido, J.; Uriarte, E.; Borges, F. Chromone: A Valid Scaffold in Medicinal Chemistry. *Chem. Rev.*, **2014**, *114*, 4960-4992.

[3] Reis, J.; Cagide, F.; Chavarria, D.; Silva, T.; Fernandes, C.; Gaspar, A.; Uriarte, E.; Remião, F.; Alcaro, S.; Ortuso, F.; Borges F. Discovery of New Chemical Entities for Old Targets: Insights on the Lead Optimization of Chromone-Based Monoamine Oxidase B Inhibitors. *J. Med. Chem.*, **2016**, 59, 5879-5893.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980). J. Reis (SFRH/BD/96033/2013) and F Cagide ((SFRH/BPD/74491/2010) were also supported by FCT and FEDER/COMPETE funds.

Short communication 4 - Working Group 1

Development of new indole derivatives as multitarget agents for the treatment of neurodegenerative diseases

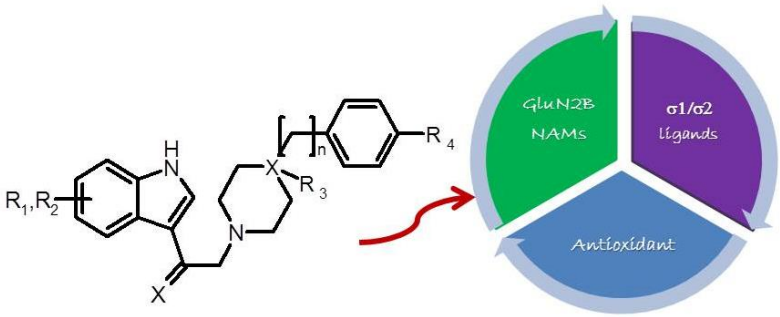
Maria Rosa Buemi,[a] Laura De Luca,[a] Stefania Ferro,[a] Rosaria Gitto[a]

[a]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali (CHIBIOFARAM) Polo Universitario SS. Annunziata, Università di Messina, Viale Annunziata I-98168 Messina, Italy*

mbuemi@unime.it

The imbalance in neurotransmitter receptor systems, free radicals and dysregulation of calcium homeostasis are multiple factors contributing neurodegenerative disorders. So the polypharmacology approach can be considered a promising strategy for the treatment of these pathologies. Negative allosteric modulators (NAMs) of NMDA receptor (NMDAR) and ligands of sigma (σ1/σ2) receptors emerged as a promising targets for neuroprotective agents. Specifically, NAMs bind to the so-called ifenprodil site and exert a blockade of NMDAR activation, thus reducing the calcium mediated excitotoxic cascade. We have previously identified a class of indoles as potent ligands of NMDAR containing GluN2B subunit (1,2). They proved to reduce NMDA mediated current in patch clamp experiments and show anticonvulsant efficacy (2). To obtain multi-target agents able to bind the GluN2B subunit as well as other receptor system involved in calcium homeostasis, we designed a series of new indoles targeting σ1/σ2 receptors. Moreover, we have introduced hydroxyl groups to produce antioxidant effects that has been tested by means of ABTS method (3).

As a continuation of our efforts to identify multi-target neuroprotective agents, we carried out a structure-based design newer indole derivatives. Specifically, the designed compounds are characterized by several chemical features modulating the interaction with selected biological targets implicated in neurodegeneration process.



References

[1]Gitto, R.; De Luca, L.; Ferro, S.; Buemi, M.R.; et al.. Synthesis and biological characterization of 3-substituted-1H-indoles as ligands of GluN2B-containing N-methyl-D-aspartate receptors. *J. Med. Chem*., **2011**, 54, 8702-8706.

[2] Buemi M.R.; De Luca L.; Chimirri A.; Ferro S.; Gitto R., et al.. "Indole derivatives as dual-effective agents for the treatment of neurodegenerative diseases: synthesis, biological evaluation, and molecular modeling studies"; *Bioorg Med Chem.;* **2013,** 21,(15), 4575-80.

[3]Gitto R.; De Luca L.; Ferro S.; Scala A., et al.. "From NMDA Receptor Antagonists to Discovery of Selective σ2 Receptor Ligands"; *Bioorg Med Chem;* **2014**, 22, 393-397.

Short communication 5 - Working Group 1

Design, synthesis and biological evaluation of novel 1-benzylpyrrolidin-3-amine derivatives as potential inhibitors of beta-secretase and cholinesterases

Wichur Tomasz,[a] Godyń Justyna,[a] Panek Dawid,[a] Więckowska Anna,[a] Jończyk Jakub,[a] Bajda Marek, [a] Malawska Barbara[a]

[a] *Department of Physicochemical Drug Analysis, Jagiellonian University Collegium Medicum, Kraków, Poland*

tomasz.wichur@doctoral.uj.edu.pl

Among patients aged 65 and older, Alzheimer's disease (AD) is the fifth leading cause of death. This fatal, neurodegenerative disease has complex nature with many factors involved in its pathogenesis. There are several well-known hallmarks of AD, which include abnormal proteins like amyloid-β plaques and neurofibrillary tangles. The mentioned proteins are of great interest to researchers as biological targets for new anti-Alzheimer's agents.[1] One of the strategies used in the search for novel therapies for AD is multi-target-directed ligand (MTDL) strategy that provides compounds acting on several targets simultaneously. This approach is especially reasonable in case of multifactorial diseases like AD due to enhanced therapeutic effect associated with the use of MTDLs.[2]

The aim of our study was design, synthesis and biological evaluation of novel 1-benzylpyrrolidin-3-amine derivatives, with potential multi-target-directed inhibitory activity against beta-secretase known as beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1) and cholinesterases. Multifunctional compounds with such activity are the object of interest in our research group.[3] Hit compound AWJ-89, a derivative of 1-benzylpyrrolidin-3-amine, which shows inhibitory activity against BACE-1 (IC_{50} = 33 μM) and butyrylcholinesterase (BuChE; 49% at the screening concentration of 10 μM), was selected from our library as a starting point for development of new inhibitors. Using our experience and molecular modeling studies we designed series of new derivatives, bearing 1-benzylpyrrolidin-3-amine moiety attached to different phenyl-substituted cyclic amines and developed synthetic route to obtain them.

Design, synthesis and results of preliminary biological evaluation against cholinesterases and BACE-1 of this series of compounds will be presented.

References:

[1] Citron, M. Alzheimer's disease: strategies for disease modification. *Nat. Rev. Drug Discov.*, **2010**, *9*, 387-398.

[2] Cavalli, A.; Bolognesi, M.L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.*, **2008**, 51(3), 347-72.

[3] Panek, D.; Więckowska; A., Wichur, T.; Bajda, M.; Godyń, J.; Jończyk, J.; Mika, K.; Janockova, J.; Soukup, O.; Knez, D.; Korabecny, J.; Gobec, S., Malawska, B. Design, synthesis and biological evaluation of new phthalimide and saccharin derivatives with alicyclic amines targeting cholinesterases, beta-secretase and amyloid beta aggregation. *Eur. J. Med. Chem.*, **2016**, In Press, Accepted Manuscript.

Short communication 6 - Working Group 1

Navigation in bile acid chemical space: discovery of novel FXR and GPBAR1 modulators

Claudia Finamore,[a] Carmen Festa,[a] Valentina Sepe,[a] Adriana Carino,[b] Dario Masullo,[a] Silvia Marchianò,[b] Stefano Fiorucci,[b] and Angela Zampella[a]

[a] Department of Pharmacy, *University of Naples "Federico II", Via D. Montesano, 49, 80131, Naples, Italy*.

[b] Department of Surgery and Biomedical Sciences, *New Faculty of Medicine, P.zza L. Severi, 1, 06132, Perugia, Italy*.

claudia.finamore@unina.it

Next to their ancestral roles in lipid digestion and solubilization, bile acids (BAs), the principal constituent of bile, are today recognized signaling molecules involved in many physiological functions, and these signaling pathways involve the activation of several metabolic nuclear receptors, mainly the BA sensor FXR[1] and the dedicated membrane G-protein-coupled receptor, GPBAR1 (TGR5).[2]

In the last years, these receptors have gained increasing consideration as druggable receptors and their exogenous dual or selective regulation represents an attractive strategy in the treatment of enter-hepatic and metabolic disorders. Medicinal chemistry on bile acid scaffold has produced several derivatives modified on the side chain, in the length and in the nature of the end-group and on the tetracyclic core. Indeed, these derivatives cover the same chemical space of BAs that are intrinsically promiscuous toward FXR and GPBAR1 and therefore, with few exceptions, this kind of speculation mainly afforded dual modulators that often associate with severe side effects. In this work, the chemical diversity of available bile acid receptor modulators has been increased with the design and the synthesis of a large family of 6-ethylcholane derivatives.[3,4]

Deep pharmacological characterization resulted in the identification of potent and selective FXR and GPBAR1 agonists.

References:

[1] Makishima, M.; Okamoto, A.Y.; Repa, J.J.; Tu, H.; Learned, R.M.; Luk, A.; Hull, M.V.; Lustig, K.D.; Mangelsdorf, D.J., Shan, B. Identification of a nuclear receptor for bile acids. *Science*, **1999**, 284, 1362-1365.

[2] Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H.; Sugiyama, E.; Nakamura, T.; Itadani, H.; Tanaka, K. Identification of membrane type receptor for bile acids (M-BAR). *Biochem. Biophys. Res. Commun*. **2002**, 298, 714-719.

[3] Sepe, V.; Festa, C.; Renga, B.; Carino, A.; Cipriani, S.; Finamore, C.; Masullo, D.; Del Gaudio, F.; Monti, M.C.; Fiorucci, S.; Zampella, A. Insight on FXR selective modulation. Speculation on bile acid chemical space in the discovery of potent and selective agonists. *Scientific Reports,* **2016**, 6, 19008.

[4] Finamore, C.; Festa, C.; Renga B.; Sepe, V.; Carino, A.; Masullo, D.; Biagioli, M.; Marchianò, S.; Capolupo, A.; Monti, M.C.; Fiorucci, S.; Zampella, A. Navigation in bile acid chemical space: discovery of novel FXR and GPBAR1 ligands. *Scientific Reports*, **2016**, 6, 29320.

Short communication 7 - Working Group 1

Synthesis of triclosan derivatives and their biological activity

Rudolf Vosátka,[a] Martin Krátký,[a] Klára Konečná,[b] Jarmila Vinšová[a]

[a] *Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic.*

[b] *Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic.*

vosatkar@faf.cuni.cz

Introduction

Tuberculosis (TB) represents one of the leading causes of morbidity and mortality worldwide. Development of new potential drugs is essential because of the existence of latent TB and development of drug-resistant TB forms (multidrug-resistant TB, extensively drug-resistant TB and recently reported totally drug-resistant TB).[1,2)] Triclosan (TRC) is a broad spectrum antibacterial agent used in household products. Triclosan has been shown to inhibit InhA, an essential enoyl acyl carrier protein which leads to the lysis of *Mycobacterium tuberculosis*.[3)] *Staphylococcus aureus* (including methicillin resistant *Staphylococcus aureus*- MRSA) and multidrug-resistant Gram-negative cocci are very effective opportunistic pathogens which are able to acquire resistance against antimicrobial drugs.[4)] Esterification of triclosan to form its prodrugs can produce compounds with improved properties – enhanced bioavailability or absorption, higher activity and/or lower toxicity. Acids of various structural types were used and screened.

Experimental methods

We used two synthetic procedures to obtain desired esters. The first pathway consists in the reaction of triclosan (1 eq.) with various acyl chlorides (1.3 eq.) in presence of triethylamine (1.5 eq.). The second approach for the preparation of the triclosan esters is the Steglich esterification using EDC. Synthesized derivatives were evaluated for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$, *M. avium* and two strains of *M. kansasii* and also against Gram-positive a Gram-negative strains (*S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus epidermidis*, *Enterococcus sp.*, *E. coli*, *Klebsiella pneumoniae* , extended-spectrum β-lactamases (ESBL)-positive *K. pneumoniae* and *Pseudomonas aeruginosa*) and fungi (yeasts *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *Trichosporon asahii* and moulds *Aspergillus fumigatus*, *Absidia corymbifera* a *Trichophyton mentagrophytes*).

Results

We prepared 26 triclosan esters based on various aliphatic, cycloaliphatic, aromatic and heteroaromatic acids. 5-Chloro-2-(2,4-dichlorophenoxy)phenyl 4-bromobenzoate (**1**) showed the best *in vitro* activity with minimum inhibitory concentrations MIC=16 μM against *Mycobacterium tuberculosis* $H_{37}Rv$. Against atypical mycobacterial strains had the best activity 5-chloro-2-(2,4-dichlorophenoxy)phenyl isonicotinate (**2**). The MIC of **2** was similar for *M. kansasii* 6509/96 and better for *M. avium* and *M. kansasii* 235/80 in comparison with INH (*Tab. 1.*). The best activity against Gram-positive and Gram-negative bacterial strains showed esters **2**, 5-chloro-2-(2,4-dichlorophenoxy)phenyl propiolate (**3**), 5-chloro-2-(2,4-dichlorophenoxy)phenyl 2-(4-oxo-2-thioxothiazolidin-3-yl)acetate. Worth to note that the compound **3** were effective against MRSA with MIC=0.49 μM. The compound **3** and 5-chloro-2-(2,4-dichlorophenoxy)phenyl pyrazine-2-carboxylate exhibited the best antifungal action.

Code	**MIC** [μM]									
	M. tuberculosis 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96		
	14 d	21 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
1	**16**	32	62.5	62,5	**16**	32	32	**16**	**16**	32
2	32	32	32	32	**8**	**16**	32	**8**	**8**	**16**
3	32	32	62.5	62.5	**16**	32	32	**16**	32	32
TRC	16	32	32	32	8	16	32	8	8	8
INH	0.5	0,5	>250	>250	>250	>250	>250	4	8	8

Table 1: The most active derivatives against Mycobacterium.

Conclusions

The *in vitro* evaluation of 26 triclosan-based esters showed promising antimycobacterial, antibacterial and antifungal activity. In general were the most effective compounds **3** and **2**, where we register broad spectrum of activity against evaluated organisms (for example mycobacterium strains, MRSA or general staphylococcus strains). The further research of the most active analogues will continue, particularly with regard to their cytotoxicity for mammalian cells.

Conflict of interest: None

References:

[1)] World Health Organization, Global tuberculosis report 2015. http://www.who.int/tb/publications/global_report/en/ (1. 9. 2016)

[2)] Krátký M.; Vinšová, J. Pokroky ve vývoji antituberkulotik působících na multilékově rezistentní kmeny. *Chem. Listy.,* **2010**, *104*, 998-1005.

[3)] Stec, J.; Vilcheze, C.; Lun, SC.; Perryman, AL.; Wang, X.; Freundlich, JS.; Bishai, W.; Jacobs, WR.; Kozikowski, AP. Biological Evaluation of Potent Triclosan-Derived Inhibitors of the Enoyl-Acyl Carrier Protein Reductase InhA in Drug-Sensitive and Drug-Resistant Strains of *Mycobacterium tuberculosis*. *ChemMedChem.,* **2014**, *9*, 2528-2537.

[4)] Mendem, SK.; Gangadhara, TA.; Shivannavar, CT.; Gaddad, SM. Antibiotic resistance patterns of Staphylococcus aureus: A multi center study from India. *Microb. Pathog.,* **2016**, *98*, 167-170.

Short communication 8 - Working Group 1

Isatin-thiazolidone hybrids as DNA G-Quadruplex selective stabilisers: a new potential approach to multitarget anticancer agents

Claudia Melis,[a] Rita Meleddu,[a] Simona Distinto,[a] Giulia Bianco,[a] Filippo Cottiglia,[a] Stefano Alcaro,[c] Claudia Sissi,[b] Elias Maccioni[a]

[a]*Dept. of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy*

[b]*Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, v. Marzolo 5, 35131,Padova, Italy*

[c]*Dipart. di Scienze della Salute, Università degli Studi "Magna Græcia" di Catanzaro, Viale Europa, 88100 Catanzaro, Italy*

claudia.melis@unica.it

G-quadruplex (G-4) is a sequence depending DNA structural arrangement assumed by Guanine-rich sequences and stabilized by the formation of guanine tetrads through eight Hoogsteen hydrogen bonds. This arrangement is further stabilized by the presence of monovalent metal ions.[1] Interestingly, these arrangements are frequent in strategic position of human genome, such telomeric ends of chromosomes, promoter regions of proto-oncogene sequences, introns, and the immunoglobulin switch regions.[2] Therefore, the stabilization of G-4 DNA structures by small molecules has emerged as a promising strategy for the development of anticancer drugs exhibiting less adverse effects, by a selective stabilization of G-4 over the duplex DNA.[1-2] To achieve this goal, a new series of isatin-thiazolidinone hybrids were designed and synthesized considering both their drug-like profile and ability to interact with G4-DNA.

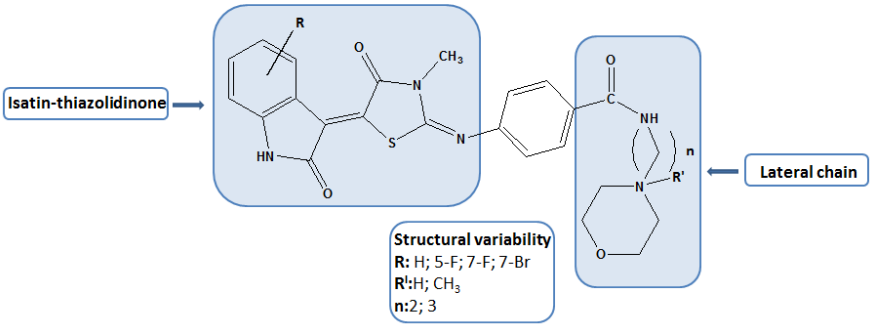


Figure 1: General structure of the isatin-thiazolidinone derivatives.

Our preliminary results indicate that this scaffold specifically stabilizes the parallel topology of promoter G4-DNA(c-MYC, c-KIT1 and c-KIT2) with good selectivity against duplex DNA. In conclusion, isatin hybrids compounds were confirmed as interesting scaffolds for the design of multi-target directed small molecules.

References:

[1] Neidle, S. Quadruplex Nucleic Acids as Novel Therapeutic Targets. *J. Med. Chem.*, **2016**, *59*(13), 5987-6011.

[2] K. V. Diveshkumar, S. Sakrikar, F. Rosu, S. Harikrishna, V. Gabelica. P.I. Pradeepkumar. Specific Stabilization of c‑MYC and c‑KIT G-Quadruplex DNA Structures by Indolylmethyleneindanone Scaffolds. *Biochemistry.*, **2016**; 55(25), 3571-85.

Short communication 9 - Working Group 1

Interaction of ATP-competitive DNA gyrase inhibitors with the cellular chaperone HSP90 as a potential pathway to inhibit viral replication

Tihomir Tomašič,[a] Katja-Emilia Lillsunde,[b] Päivi Tammela,[b] Danijel Kikelj[a]

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

[b] *Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland*

tihomir.tomasic@ffa.uni-lj.si, danijel.kikelj@ffa.uni-lj.si

Human heat shock protein 90 (Hsp90), bacterial DNA gyrase (GyrB) and topoisomerase IV (ParE) belong to the GHKL ATPase superfamily of enzymes. Because of the structural similarities between the ATP-binding sites of GyrB and ParE, dual targeting is possible in most bacteria, which prolongs the onset of resistance and makes them attractive targets for antibacterial drug discovery. Hsp90 is a chaperone with ATPase activity, which ensures the correct folding and assembly of many proteins in cells, and is an important target for anticancer drug development. In addition, it has been recently shown that human Hsp90 is a key factor enabling efficient virus replication. In addition to needing the chaperones for protein processing, viruses are also capable of optimizing the cellular microenvironment for virus replication through chaperone regulation.

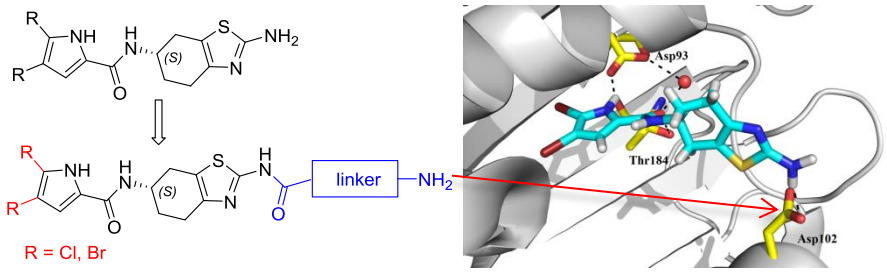


Figure 1: Design strategy of the novel inhibitors of bacterial DNA gyrase, topoisomerase IV and human Hsp90.

A library of synthetic analogues of marine alkaloid oroidin was screened against replicon models of hepatitis C virus (HCV). Four compounds were found to inhibit the HCV replicon (IC_{50} 2.0-11 µM), being more potent than drug ribavirin (IC_{50} 58 µM) and showing low cytotoxicity (CC_{50} 79-120 µM) in HCV replicon. These belong to the 4,5,6,7-tetrahydrobenzo[1,2-d]thiazole class of compounds originally designed to target the ATP-binding site of bacterial DNA gyrase. Compound binding to Hsp90 was evaluated through microscale thermophoresis and molecular modelling, which confirmed our hypothesis of compounds' interaction with Hsp90 (K_d 18-79 µM) as a basis for their antiviral activity. Structure-based optimization of initial hits resulted in compounds with improved Hsp90 (K_d 4.2-158 µM) and anti-HCV (IC_{50} 1.1-20 µM) activities. The presented novel structural class of small-molecule Hsp90 inhibitors has potential for development of antiviral agents.

References:

[1] Lillsunde, K.-E.; Tomašič, T.; Kikelj, D.; Tammela, P. Marine alkaloid oroidin analogues with antiviral potential: a novel class of synthetic compounds targeting the cellular chaperone Hsp90. *Submitted.*

Short communication 10 - Working Group 1

Rational Design and Synthesis of Novel Amidine-Based Scaffolds as BACE1 Inhibitors

Myriam Ciordia Jiménez,[a,b] Laura Pérez Benito,[b] Natalia Mateu,[c] Óscar Delgado,[b] María Sánchez Roselló,[c] Santos Fustero,[c] Gary Tresadern,[b] Beatriz de Pascual-Teresa,[a] Andrés A. Trabanco[b]

[a] *Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU San Pablo, 28668 Boadilla del Monte, Madrid, Spain.*
[b]*Neuroscience Medicinal Chemistry, Janssen Pharmaceutical Research & Development, 45007 Toledo, Spain.*
[c]*Departamento de Química Orgánica, Universidad de Valencia, 46100 Burjassot, Valencia, Spain.*

myriam.ciordia@gmail.com

Alzheimer's disease (AD) is a progressive neurodegenerative disorder considered to be the most common cause of dementia.(1) Since the discovery that BACE1 is essential for processing amyloid precursor protein (APP), this enzyme has become the prime target for the treatment of AD. However, after more than 15 years of research BACE1 has proven to be an exceptionally difficult target, where identifying small-molecule inhibitors which combine good pharmacological and pharmacokinetic properties has been a challenge.(2)

Amidine- or guanidine-containing heterocycles were recently discovered to establish an ideal hydrogen-bonding network with the catalytic dyad of the enzyme. However, control of the basicity of the amidine moiety in a BACE1 inhibitor is essential not only to achieve a suitable interaction with the catalytic aspartates but also to improve central penetration.(3) The pKa of the amidine could be modulated by the introduction of electron withdrawing groups (EWG) in the warhead. Often, fluorine or polyfluoro alkyl groups have been used, as these functional groups also have a marked effect on physicochemical properties of molecules.

We report the novel one-pot synthesis of a new family of quaternary fluorinated pyrrolidones starting from unprotected aromatic amino esters using as key synthetic step a tandem Michael addition/cyclization reaction as well as its further derivatization into novel mono and spirocyclic amidine-based BACE1 inhibitors with tunable pKa. Docking and FEP studies allowed us to optimize the structure of the spirocyclic series, improving their potency significantly.

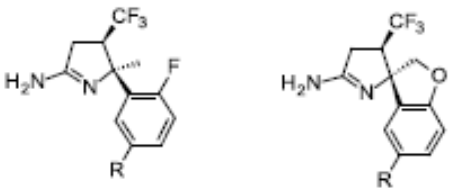


Figure 1: General structure of the target amidine-containing BACE1 inhibitors.

References:

[1] Alzheimer's Association. 2014 Alzheimer's disease facts and figures. *Alzheimers Dement.* **2014**, *10*, 47-92.

[2]Li, Q. ; Fuller, S. J.; Beyreuther, K.; Masters, C.L. The amyloid precursor protein of Alzheimer's disease in human brain and blood. *Journal of Leukocyte Biology.* **1999**, *66*, 567-574

[3]Tresadern, G.; Delgado, F.; Delgado, O.; Gijsen, H.; Macdonald, G. J.; Moechars, D.; Rombouts, F.; Alexander, R.; Spurlino, J.; Van Gool, M.; Vega, J. A.; Trabanco, A. A. Rational design and synthesis of aminopiperazinones as β-secretase (BACE) inhibitors. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 7255-7260.

Short communication 11 - Working Group 3

Let's develop together an exchange virtual compounds computational platform: Updates!

Carmine Talarico,[a] Francesco Ortuso,[a] Stefano Alcaro[a]

[a] *Dept. of Health Sciences, University "Magna Graecia" of Catanzaro, Campus "S. Venuta", Catanzaro, Italy*

talarico_c@unicz.it

The development of Mu.Ta.Lig chemotheca it's moving on. Further code has been written to make users registration and the compounds management (upload and search) procedures, as simple as possible. An early-alpha version of the web site allows Users registration and login, compounds upload and a still basic search services. Before to be released, beta-testing are still running and potential users suggestion will be collected.

At the registration, the users will obtain, by email, a "one-time password" valid for the first login session only. At first authenticate session, users will change their password that will be stored in encrypted (MD5) format. As a consequence, the definitive password will can not be retrieved and a "Forget password" procedure has been defined.

Registered users can upload their own molecules and/or experimental data deciding the corresponding access policy. Compounds activity data will available to registered user only. If required in upload procedure, an automatic email will inform the data owner in case of download. The email address of the "downloader" will be included into the message. In all cases downloads will be registered.

Molecules can be materially obtained from owners, after agreement between applicant and owner without intermediation by Chemotheca management.

Chemotheca will require to "Downloaders" to update information related to the obtained compounds, if availables.

In order to increase the impact of Chemotheca on international scientific scenario, Guest users will be allowed to search a "public area" of the Database where will be stored, according to the corresponding owner, some compound structure but not activity (both experimental and theoretical) data.

The Chemotheca's expected results, will be achieved when large number of high quality compounds will be uploaded, classified and when scientific collaboration among Mu.Ta.Lig. COST ACTION (CA 15135) participants will be promoted.

Short communication 12 - Working Group 3

Natural Compounds from NuBBE database as Inhibitors of Zika Virus - E Protein

Tomislav Kostevc, Črtomir Podlipnik

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113,

1000 Ljubljana, Slovenia

crtomir.podlipnik@fkkt.uni-lj.si

Zika virus (ZIKV) is member of the family of *Flaviviridae,* which includes the West Nile virus, dengue virus, yellow fever virus among others. The virus is named after the Uganda's forest where the virus was isolated in samples taken from rhesus monkey in 1947. First large Zika outbreak in humans in the Pacific Island of Yap. Prior to this, no outbreaks and only 14 cases of human ZIKV disease had been documented anywhere in the world. An estimated 73% of Yap residents are infected with this highly infective virus. In 2015 the number of human infections with this mosquito-borne virus dramatically increase in Latin America, especially in Brazil. Zika is spread mostly by the bite of an infected *Aedes* species mosquito. However, there is the evidence of some non-vector transmission of the disease via sexual contacts. ZIKV infections are manifested with symptoms of fever, joint and muscle pain, rash and conjucticivitis. Infection normally last up to one week and people usually don't get sick enough to search for medical assistance. Pregnant women are the most affected to threatening action of Zika. It is known that this virus can cause a birth defect of the brain called microcephaly. The Gullian-Barre syndrome, which is an uncommon sickness of the nervous system, is probably another complication that may be a consequence of the infection with the ZIKV. The best way to prevent Zika in threatening areas is to protect yourself and your family from mosquito bites. Unfortunately, there is no vaccine or drug to prevent Zika.[1,2]

In present study we targeted a ZIKV Envelope (E) protein, which is responsible for virus entry and it is also a major target of neutralizing antibodies. Based on the model of ZIKV E protein (PDB-ID:5JHM)[3] [3] we have perfomed Schrödinger's Virtual Screening Workflow [4] to extract 25 of the most promising compounds from the NuBBE database, which is a virtual database of natural products and derivates from Brazilian biodiversity.[5] Derivate of Quercetin isolated from Maytenus aquifolium (Celastraceae) with NuBBE_ID:361 has the lowest GlideXP score (-13.5 kcal/mol) among all studied compounds.

Since this presentation will be part of WG3 Section we will also give a short review of Natural Products Database from the Biodiversity of Brazil (NuBBE) with aspects of it's development and usefulness.

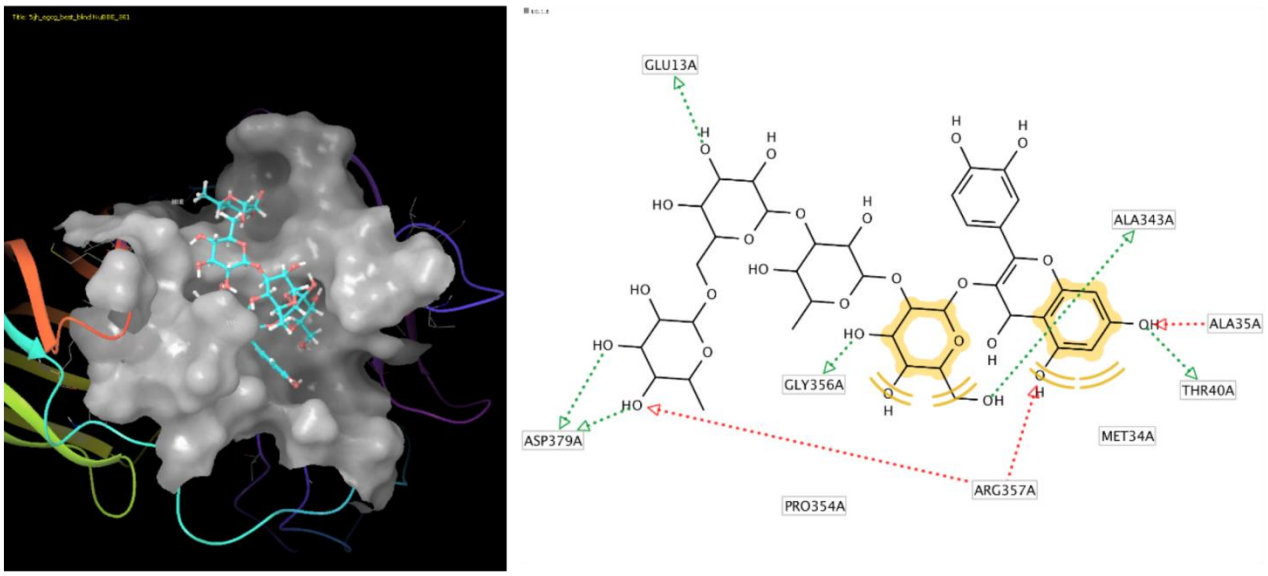


Figure 1: Interaction between Ligand NuBBE_361 and ZIKV E protein.

Key words: Molecular Docking, Structure Based Drug Design, Zika Virus, Natural Compounds

References:

[1]URL: https://www.cdc.gov/zika/ [accessed 14/07/16]

[2]Mlakar, J.; *et al*. Zika Virus Associated with Microcephaly. *N Engl J Med*, **2016**, *374(10)*, 951-8.

[3]Dai, L.; *et al.* Structures of the Zika Virus Envelope Protein and Its Complex with a Flavivirus Broadly Protective Antibody. *Cell Host & Microbe*, **2016,** *19*, 696-704.

[4]URL:https://www.schrodinger.com/suites/small-molecule-drug-discovery-suite [accessed 14/07/2016]

[5]Valli, M.; *et al.* Development of a Natural Products Database from the Biodiversity of Brazil. *J. Nat. Prod.*, **2013**, 76 (3), 439-44.

Short communication 13 - Working Group 4

A computational multitarget screening for the in silico identification of bioactive compounds

Roberta Rocca,[a] Annalisa Maruca,[a] Anna Artese,[a] Isabella Romeo,[a] Giosuè Costa,[a] Federica Moraca,[a] Carmine Talarico,[a] Gudrun Englmaier,[b] Judith Rollinger,[b] Stefano Alcaro[a]

[a] *Department of Health Sciences, University "Magna Græcia" of Catanzaro, Viale Europa, 88100 Catanzaro, (Italy).*
[b] *Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna (Austria).*

rocca@unicz.it

Today, polyphamacology is recognized as a new valuable opportunity for drug discovery and development[1]. In the context of polypharmacology and off-target interactions, structure-based methods of protein function prediction gained an important role[2]. Indeed, unexpected ligand-target interactions can be discovered by cross-docking libraries of compounds against known different proteins[3]. With the aim to identify new potential multi-target lead compounds, in this work we screened a database of natural molecules extracted from plants (such as *Atropa belladonna*, *Allium cepa*). Among the 10 best multi-target *hits* (**Figure 1**), some compounds, derived from *Cannabis sativa L.*, resulted very promising, since they showed a good theoretical affinity *versus* different targets of medicinal chemistry interest, proving a potential polypharmacological profile for the design of new multi-target agents.

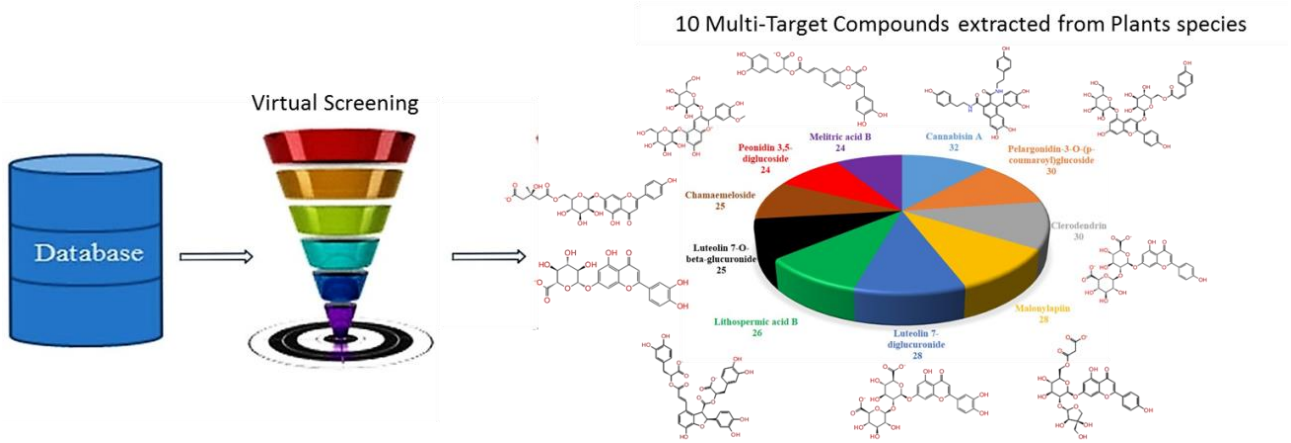


Figure 1: Virtual Screening workflow and the 10 best multi-target *hits*.

References:

[1] Rastelli, G.; Pinzi, L. Computational polypharmacology comes of the age. *Front Pharmacol,* **2015**, *6*, 157.

[2] Laskowski, R.A.; Watson, J.D.; Thornton, J.M. ProFunc: a server for predicting protein function from 3D structure. *Nucleic Acids Res,* **2005**, *33,* 89-93.

[3] Favia, A.D.; Nobeli, I.; Glaser, F.; Thornton, J.M. Molecular docking for substrate identification: the short-chain dehydrogenases/reductases. *J Mol Biol,* **2008**, *375,* 855-874.

Short communication 14 - Working Group 4

Validation of Docking and Dynamic protocol for the study of a new class of Oxadiazoles able to inhibit MAO-B and to prevent Neuronal Oxidative damage

G. Bianco,[a] P. Cossu,[a] S. Distinto,[a] R. Meleddu,[a] A. Arridu,[a] M. Yanez,[b] R. Cirilli,[c] F. Ortuso,[d] S. Alcaro,[d] E. Maccioni[a]

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

[b] *Facultad de Farmacia, Departamento de Farmacología, Universidad de Santiago de Compostela, Campus Vida, La Coruña, 15782 Santiago de Compostela, Spain*

[c] *Dipartimento del Farmaco, Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Roma, Italy*

[d] *Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", Viale Europa, 88100 Catanzaro, Italy*

giuliabianco@unica.it

The pivotal role of type B Mono Amino Oxidase (MAO-B) in the metabolism of neurotransmitters made this enzyme a very interesting target in medicinal chemistry. MAO-B expression increases with age, causing a decreased availability of dopamine and an increased degree of oxidative damage to neuronal cells. This two factors can lead to neurodegeneration and Parkinson's disease. Therefore, the inhibition of MAO-B is interesting for a multi-target approach because by blocking its activity both the neuronal oxidative damage is prevented and the level of neurotransmitter is increased. For this reasons selective MAO-B inhibitors have received considerable attention in the treatment of several neurological and neurodegenerative pathologies.[1] Our efforts lead to the identification of new compounds, based on the 3-acetyl-2-dichlorophenyl-5-aryl-2,3-dihydro-1,3,4-oxadiazole chemical scaffold, able to inhibit MAO-B in the nanomolar range. The enantiomers of most promising derivatives were separated by enantioselective HPLC and *in vitro* evaluated. Finally, docking experiments coupled to molecular dynamics (MD) simulations protocols, were first validated and then applied for understanding the putative MAO-B binding modes of the new compounds providing detailed information for further structural optimization.[2]

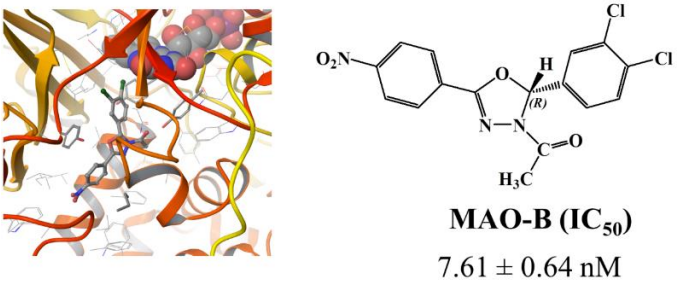


Figure 1: Putative binding mode of the best synthesized compound.

References:

[1] Youdim, M. B. H.; Edmondson, D.; Tipton, K. F. *Nat. Rev. Neurosci.* **2006**, *7*, 295.

[2] Distinto, S., Meleddu, R., Yanez, M., Cirilli, R., Bianco, G., Sanna, M. L., Arridu, A., Cossu, P., Cottiglia, F., Faggi, C., Ortuso, F., Alcaro, S., Maccioni, E. *Eur. J. Med. Chem*. **2016**, *108*, 542-552.

Short communication 15 - Working Group 2

Dual enzyme inhibitors: a promising multi-target approach to cancer therapy

R. Meleddu,[a] S. Distinto,[a] C. Melis,[a] G. Bianco,[a] F. Cottiglia,[a] C.T. Supuran[b] and E. Maccioni[a]

[a] *Dept of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy*

[b] *Dipt of NEUROFARBA, Sezione di Scienze Farmaceutiche, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy*

rita.meleddu@unica.it

A promising approach to obtain multi-target inhibitors for the therapy of multifactorial disease could be represented by the design of dual enzyme inhibitors. In this respect the identification of cyclooxygenase and human carbonic anhydrase inhibitors is a very attractive challenge in the design of anti-cancer agents. [1-4] Tumours are characterised by both inflammation and hypoxia. These conditions lead to an altered extracellular pH that, in turns, favour adjacent tissues invasion by cancer cells. In this study, starting from a common active fragment [5] we have designed and synthesised new molecular entities that are potential dual inhibitors of both target enzymes. On this basis, a series of differently substituted benzene sulphonamides has been designed, synthesised and evaluated for their biological activity on the two target enzymes. [6]

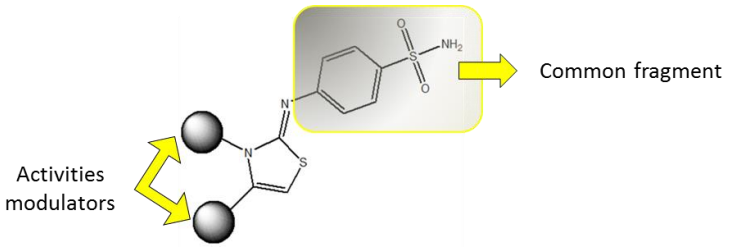


Figure 1: general structure of the compounds under investigation.

References:

[1]Pedro M., Baeza S., Escudero M. T., Dierssen-Sotos T., Gómez-Acebo I., Pollán M., Llorca J. Effect of COX-2 inhibitors and other non-steroidal inflammatory drugs on breast cancer risk: a meta-analysis. Breast Cancer Res Treat, 2015 , 149(2), 525-36.

[2]Harris R. E., Beebe J., Alshafie G. A. Reduction in cancer risk by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors J Exp Pharmacol, 2012; 4, 91-96.

[3]Supuran C. T., Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry Bioorg Med Chem, 2007, 15(13), 4336-50

[4]Supuran C. T. How many carbonic anhydrase inhibition mechanisms exist? J Enz Inhib Med Chem, 2016, 31(3), 345-60.

[5]Supuran C. T., Winum J. Y. Drug Design of Zinc-Enzyme Inhibitors John Wiley & Sons, 2009.

[6]Meleddu R., Maccioni E., Distinto S., Bianco G., Melis C., Alcaro S., Cottiglia F., Ceruso M., Supuran C. T. New 4-[(3-cyclohexyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamides, synthesis and inhibitory activity toward carbonic anhydrase I, II, IX, XII. Bioorg Med Chem Lett, 2015, 25, 16, 3281-3284

Short communication 16 - Working Group 2

Surface proteomics, a step to simplify the rapid prototyping of nanodrugs

Jacob Kuruvilla,[1] Ana Paula Farinha,[1] Narges Bayat,[2] and Susana Cristobal[1]

[1]*Department of Clinical and Experimental Medicine, Cell biology, Faculty of Medicine, Linköping University, Linköping, Sweden.*

[2]*Department of Biochemistry and Biophysics, Arrhenius laboratories, Stockholm University, Stockholm, Sweden.*

Susana.Cristobal@liu.se

Bioactive compound discovery could fail in the discovery phase even after an extended biological and toxicological assessment for two main reasons: i) the molecule does not produce the expected effects in the target cell or ii) the molecule is not safe for the target cell or surrounded cells. Therefore the most important step to evaluate of novel bioactive molecules is to identify their targeting capability, their uptake capability, their functionality at destiny and their safety from administration to target.

In the case of nanomaterials for nanodrugs, the challenge is not any longer to synthesize complex materials at the nano-size rather to develop alternative methodologies for its rapid and cost-efficient functional characterization. The current path from nanodrug´s discovery to nanodrug´s probed applicability is still based on: NM design, NM synthesis, classical *in vitro* toxicology assays in a case-by-case basis, and finally *in vivo* validation on animal models [1-5]. This path is costly, time-consumable and offered a low success-rate. In our lab we develop solutions based on considered the specific nano-sized properties and results from our recent studies of the nanoimpact project [6-12], and asking a very first question: *"What a cell see when meet a targeted nanomaterial?"*. We have defined that there are other four main questions to answer to predict a nanodrug´s function *in vivo* without applying neither *in vitro* assays nor *in vivo* models. Those questions are: *I) would this molecule be targeted to the destiny cell? ii) would this molecule be uptaken by the cell?: iii) would this molecule be functional and efficient?, and iv) what is the possible risk for the surrounded cells that are not the targeted cell?.* The solution that we offered could answer those questions and it is based on: i) our proteomic-based method that unable to obtain the minimal information required to answer those questions called SUSTU (surface proteomics for tracking nanoparticle targeting based on nanoparticle protein corona), ii) developing an integrated platform to enhance our capability to answer those question in the extended context of the nanodrug journey from administration-to target and finally iii) apply several iteration of the process to improve the nanodrug design-function based on the concept of " rapid prototyping of nanomaterials".

[1] Almeida, J. P., Lin, A. Y., Figueroa, E. R., Foster, A. E., Drezek, R. A. *Small* 2015, *11*, 1453-1459.

[2] Barth, B. M., E, I. A., Shanmugavelandy, S. S., Kaiser, J. M.*, et al. ACS Nano* 2011, *5*, 5325-5337.

[3] Bayat N., L. V. R., Sanchez-Dominguez M. , Lakshmanan R. , Rajarao G.K. and Cristobal S**.** *Environmental Science: Nano* 2015, *2*, 380 - 394.

[4] Bayat, N., Lopes, V. R., Scholermann, J., Jensen, L. D., Cristobal, S. *Biomaterials* 2015, *63*, 1-13.

[5] Liu, R., Jiang, W., Walkey, C. D., Chan, W. C., Cohen, Y. *Nanoscale* 2015, *7*, 9664-9675.

[6] Bayat, N., Rajapakse, K., Marinsek-Logar, R., Drobne, D., Cristobal, S. *Nanotoxicology* 2014, *8*, 363-373.

[7] Lopes, V. R., Loitto, V., Audinot, J. N., Bayat, N.*, et al. J Nanobiotechnology* 2016, *14*, 22.

[8] Rajapakse, K., Drobne, D., Kastelec, D., Kogej, K.*, et al. Nanotoxicology* 2016, *10*, 542-556.

[9] Bayat, N., Lopes, V. R., Sanchez-Dominguez, M., Lakshmanan, R.*, et al. Environ. Sci.: Nano* 2015, *2*, 380-394.

[10] Bayat, N., Lopes, V. R., Scholermann, J., Jensen, L. D., Cristobal, S. *Biomaterials* 2015, *63*, 1-13.

[11] Tedesco, S., Bayat, N., Danielsson, G., Buque, X.*, et al. Journal of Integrated OMICS* 2015, *5*, 44-57.

[12] Garcia-Sanchez, S., Bernales, I., Cristobal, S. *BMC genomics* 2015, *16*, 341.

Short communication 17 - Working Group 2

Screening the central effects of multitarget agents, H3 receptor antagonists/inverse agonists

Wieslawa Agnieszka Fogel,[a] Anna Stasiak,[a] Marek Staszewski,[b] Beata Olszewska,[b] Krzysztof Walczynski[b]

[a] *Dept of Hormone Biochemistry, Medical University of Lodz, Zeligowskiego 7/9, 90-752 Lodz, Poland.*

[b] *Dept of Synthesis and Technology of Drugs, Medical University of Lodz, Muszynskiego 1, 90-151 Lodz, Poland*

wieslawa.agnieszka.fogel@umed.lodz.pl

In the central nervous system histamine is involved in the regulation of such complex brain functions as sleep-awake cycle, cognitive processes or feeding behavior. Moreover, under life threatening conditions (hypovolemic shock, hypoglycemia, dehydration) it triggers compensatory mechanisms supporting survival (1). Histamine effects are mediated by four receptors (H1R-H4R), of them H1-H3 receptors proven to be expressed and acting in the CNS. The H3Rs function as autoreceptors or heteroreceptors, controlling the release and synthesis of histamine and/or other neurotransmitters (acetylcholine, noradrenaline, dopamine, serotonin, GABA and Glu), respectively. This enables complex interaction of histaminergic system with other neuronal systems. From therapeutic point of view it make the H3 receptor ligands an important pharmacological multitarget agents. Experimental data strongly suggest, the H3 receptor antagonists by increasing extracellular level of neurotransmitters can become useful drugs in narcolepsy, epilepsy, obesity, schizophrenia or memory and learning deficits (2). And accordingly, the first H3 ligand has recently been approved to treat narcolepsy in man (the trade name Wakix). In many laboratories works are ongoing to develop centrally acting non- toxic, non-imidazole antagonists/inverse agonists for the other diseases therapy. The data will be presented on the compounds from series of derivatives of 4-hydroxypiperidine and piperazine. Based on *in vitro* screening 5-[[1-(benzofuran-2-ylmethyl)piperidin-4-yl]oxy]-N-methyl-N-propylpentan-1-amine (BO-80), N-4-chlorobenzyl-,N-benzyl-N-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine (MST 1014, MST 1019) and N-benzyl-N-methyl-3-[2-(4-propylpiperazin-1-yl)thiazol-5-yl]propan-1-amine (RG 1011) were chosen for further in vivo pharmacological studies. The capacity to inhibit feeding was elected. Given the compound enters the CNS and block H3R it should release histamine which in turn via H1R would exert anorectic effect (1,2). Male Wistar or Lewis rats were used. The compounds were given in a daily dose of 3mg/kg, subcutaneously, for 5 days to animals kept individually in metabolic cages. As the reference compound Ciproxifan was employed. Consumption of feed and water and urine excretion were monitored daily. Rats were then sacrificed and their brain collected to measure concentrations of amine neurotransmitters by RIA or ELISA, and histamine N-methyltransferase and monoamine oxidase activities by radioizotopic assays. Of the tested compounds the best turned out to be MST1014 which had similar potency as Ciproxifan in feed consumption decrease; somewhat lower effect was found for MST1019 and still lower for MST10121. The other agents did not influence feeding. The cerebral amine concentrations and likewise, the enzyme activities showed some changes, however, statistically insignificant. Summing up the applied test helped to pick out the candidates for further development.

References:

[1] Passani, M.B.; Panula, P.; Lin, J.S. Histamine in the brain. Front Syst Neurosci. 2014, 8, 64.

[2]Khanfar, M.A.; Affini, A.; Lutsenko, K.; Nikolic, K.; Butini, S.; Stark, H. Multiple Targeting Approaches on Histamine H3 Receptor Antagonists. Front Neurosci. 2016, 10:201.

Short communication 18 - Working Group 2

Targeting metastasis-associated in colon cancer-1 (MACC1) via HGF-Met-MAPK pathway as novel predictive marker for breast cancer

Daria Ler[a] and Jasminka Mujić[b]

[a]*Dept of Laboratory medicine and Histopathology, Laboratory for Molecular Diagnostics, PZU Moja Klinika-General Hospital Sarajevo, Kranjčevićeva 12, 71 000, Sarajevo, Bosnia and Herzegovina*

[b]*Dept of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, International University of Sarajevo, Hrasnička cesta 15, 71 000 Sarajevo, Bosnia and Herzegovina*

dler@mojaklinika.ba

The variations in morphologies, its biochemical etiology and differences in metastatic behavior and in the response to therapeutic treatments make breast cancers hard to treat. Identifying risk markers and elucidating pathways responsible for breast cancer cell proliferation is important for the improvement of the risk classification in patients as well as discovery of therapeutic targets. Currently, MACC1 protein expression studies in clinical-pathologically characterized breast cancer samples was evaluated only by immunohistochemistry and showed significant MACC1 expression associated with clinical stage and lymph node metastasis. Our goal is to investigate potential role of MACC1 as an effective therapeutic target and to elucidate the possible role of MACC1 in breast cancer metastasis. Taken together, MACC1 harbors a great potential to be used in clinics as a prognostic marker for the identification of high-risk patients and could be used to predict cancer recurrence and therapy response.

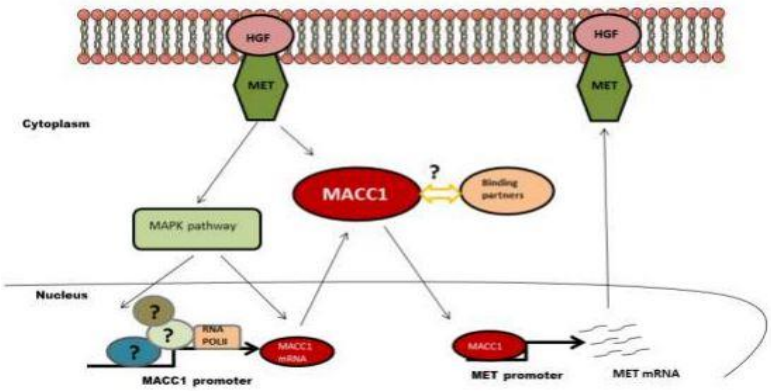


Figure 1: Schematic model of MACC1 regulating HGF signaling. HGF translocate MACC1 from the cytoplasm to the nucleus where it binds to the Met promoter. This transcriptional activation of Met, thus forms a positive feedback looping hyper activating HGF-Met pathway and thus metastasis. Furthermore MACC1 is suggested to be a downstream target of MAPK. (Adapted from Arlt et al.).

References:

[1] Arlt, F. and U. Stein, Colon cancer metastasis: MACC1 and Met as metastatic pacemakers. The International Journal of Biochemistry & Cell Biology, **2009**. 41(12): p. 2356-2359.

[2] Huang et al. Overexpression of MACC1 and its significance in human breast cancer progression. Cell & Bioscience, **2013**, 3:16.

Short communication 19 - Working Group 2

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

Tiago Silva,[a] Jorge Garrido,[b] Fernando Remião,[c] Paulo Oliveira,[d] Fernanda Borges[a]

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

[b] *Department of Chemical Engineering, Instituto Superior de Engenharia do Porto (ISEP), Instituto Politécnico Porto, Portugal*

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

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

nuno.silva@fc.up.pt

Neurodegenerative diseases (ND) are a large group of disorders of the central nervous system (CNS) with heterogeneous clinical and pathological expressions, affecting specific neuronal groups and brain signaling networks. Current single-target treatments fail to modify disease progression and are commonly associated with severe side effects. For instance, the catechol O-methyltransferase (COMT) inhibitor tolcapone used in the clinical management of Parkinson's disease (PD) was implicated in three fatal cases of fulminant hepatitis. In this context, the development of multitarget-directed drugs (MTDDs) that interact with different pharmacological targets involved in ND is currently a trending and valid strategy for the development of disease-modifying drugs for ND. If guided by accurate structure-property-activity and toxicity relationships (SPAR and SPTR, respectively), the rational design CNS drugs can overcome the two main gatekeepers of the brain, the blood-brain barrier (BBB) and P-glycoprotein (P-gp), and lead to effective and safe disease-modifying MTDDs. To this end, we focused our drug design strategy on hydroxycinnamic acids (HCAs), a class of naturally-occurring antioxidants with several in vitro pharmacological activities, including neuroprotection. Accordingly, a small library of lipophilic HCA derivatives was designed to meet the main pharmacokinetic requirements to cross the BBB, interact with multiple targets within the CNS and mitigate the formation of reactive metabolites. We successfully identified lipophilicity, redox potential and chemical structure as the main descriptors of pharmacological activity, BBB permeability and hepatotoxicity risk. Due to its BBB permeability, transition metal chelation capacity, potent inhibition of COMT activity and tau aggregation, increased safety over tolcapone and straightforward potential for chemical modification, CNCAPE can be proposed as a lead for future optimization and development of MTDD candidates for ND. A general overview of the synthetic strategies pursued in this work, as well as the results of physicochemical properties, pharmacological and toxicological studies 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).

Short communication 20 - Working Group 2

Discovery of a natural ester of ferulic acid as single inhibitor of HIV-1 RT and synthesis of its dual inhibitors analogues

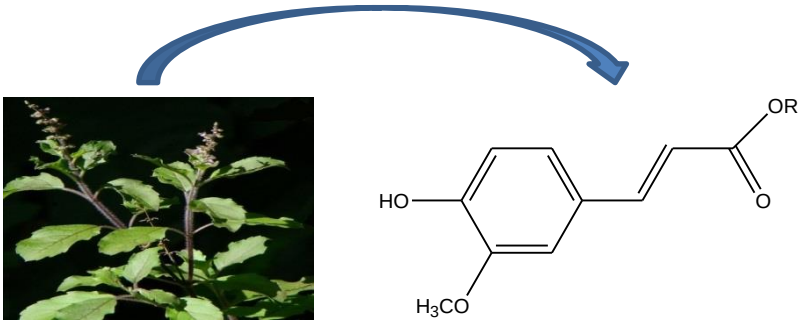
Vijay Sonar,[1] Elias Maccioni,[1] Simona Distinto,[1] Angela Corona,[2] Enzo Tramontano,[2] Filippo Cottiglia[1]

[1]*Department of Life and Environmental Sciences, Drug Sciences Section, University of Cagliari, via Ospedale 72, Cagliari 09124, Italy*

[2]*Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, Monserrato 09042 Italy*

sonarvijay21@gmail.com

The HIV-1 Reverse Transcriptase (RT) is a validated and deeply explored biological target for the treatment of AIDS. HIV-1 RT catalyses the reverse transcription process which consists of the conversion of a single strand viral RNA into a double strand viral DNA via the formation of a RNA-DNA hybrid. To perform this activity two main catalytic functions are associated in one enzyme, DNA polymerase (DP) and ribonuclease H (RNase H). However, despite the fact that both functions are validated drug targets, since also RNase H function is essential for the reverse transcription process [1], no inhibitor that target this enzymatic activity has been introduced in therapy until now [2]. Clearly, the development of compounds inhibiting both activities would have several advantages, leading to a complete block of RT functions, new favourable drug resistance profiles, reduction of combined drugs and of toxic side effects. *Ocimum sanctum,* also known as *Tulsi,* native of India and is being traditionally used medicinal plant [3]. In our continuous search for plant bioactive compounds, we have found that DCM extract of *O. sanctum* leaves showed good inhibitory activity (IC_{50}=4.2 µg/ml) towards HIV-1 RT-associated RNase H function. Bioguided isolation of the extract gave a feruloyl ester which was able to inhibit RNase H function with a IC_{50} value of 4.73 µg/ml and due to the synthetic accessibility of this secondary metabolite, a structure-activity relationship study was carried out. A series of analogues were synthesized and some compounds displayed a strong inhibitory activity towards both RT-associated functions, ribonuclease H and DNA polymerase.



References:

1. Schatz O.; Cromme F. V.; Grüninger-Leitch F.; Le Grice S. F. J. Point mutations in conserved amino acid residues within the C-terminal domain of HIV-1 reverse transcriptase specifically repress RNase H function. *FEBS Lett.* **1989**, *257*, 311-314.
2. Corona A.; Esposito F.; Tramontano E. Can the ever-promising target HIV reverse transcriptase-associated RNase H become a success story for drug development? *Future Vir.* **2014**, *9*, 445-448.
3. Mondal S. The science behind sacredness of Tulsi, *Indian J. Physiol. Pharmacol.* **2009**, *53*, 291-306.

Short communication 21 - Working Group 2

Multitargeting prosurvival pathways in human leukemic cells by metformin and thymoquinone

Mirza Suljagic,[a] Una Glamoclija[a]

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

msuljagic@ius.edu.ba

Generation of resistance to current treatment options is common problem in the therapy of many malignancies. Metformin (Met) and thymoquinone (TQ) are two molecules which have proven safety profile and represent potential candidates for treatment of hematological malignancies. We evaluate the effects of TQ and Met on inhibition of prosurvival pathways which are commonly activated in NHLs and CML: PI3K/Akt/mTor and NfkB pathway.

It was shown that tumor cells with high basal levels of pAkt are resistant to Met induced apoptosis therapy. We found that both, metformin and TQ, show inhibitory effects on the survival of studied cancer cell lines. Further we show how TQ, metformin and their combination affect apoptosis, proliferation, and metabolic properties of CML and lymphoma cell lines (GC and ABC derived) and explore whether TQ would be able to inhibit sustained Akt activity and if this inhibition would be sufficient to render leukemic cells sensitive to metformin-induced apoptosis.

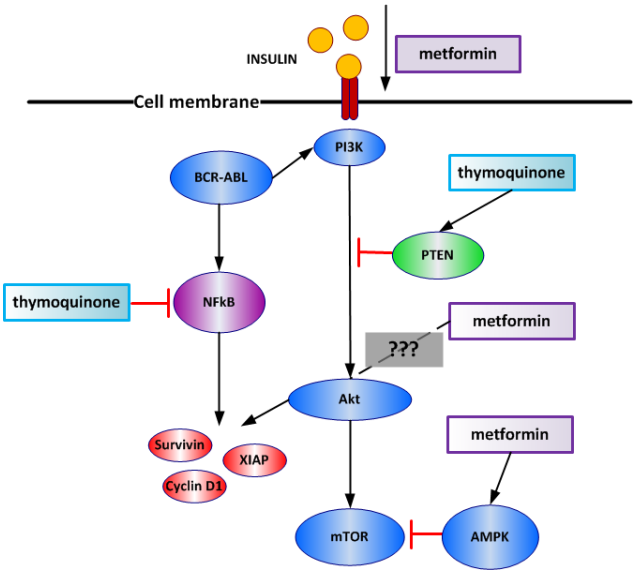


Figure 1: TQ and Met Targets.

Short communication 22 - Working Group 2

Targeting dietary antioxidants to mitochondria as a therapeutic solution for mitochondrial oxidative stress related diseases

José Teixeira,[a,b] Farhan Basit,[c] Fernando Cagide,[a] Sofia Benfeito,[a] Werner J.H. Koopman,[c] Paulo J. Oliveira,[b] Fernanda Borges[a]

[a]CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto 4169-007, Portugal; [b]CNC – Center for Neuroscience and Cell Biology, UC-Biotech Building, Biocant park – Cantanhede, University of Coimbra, Portugal; [c]Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6500HB Nijmegen, The Netherlands.

jos@fc.up.pt

Oxidative stress results from local or global imbalances towards a more oxidizing environment that leads to a disruption of redox signaling control and/or molecular damage. Given the importance of mitochondria to cellular redox signaling, those organelles are a promising target for pharmacological interventions aimed to decrease oxidative damage. Phenolic acids such as hydroxycinnamic (HCA) are natural regulators of the cellular redox status and have pharmacological interest due to their intrinsic antioxidant properties. Despite HCAs biological potential, their usefulness as therapeutic agents is limited mainly due to their low lipophilicity and druggability. Hypothesizing that mitochondriotropic antioxidants may overcome the limitations associated with HCAs, we have developed a new familiy of mitochondriotropic antioxidants (AntiOxCINs).

The data obtained along our study showed that all AntiOxCINs can accumulate in the mitochondria driven by the mitochondrial transmembrane electric potential ($\Delta\Psi m$). In addition, AntiOxCINs prevented mitochondrial lipid peroxidation caused by different oxidative insults. Importantly, AntiOxCINs toxicity profile was found to be dose-dependent and was only relevant for concentrations above those needed to exert their antioxidant activity. Our data also showed that AntiOxCINs displayed a low toxicity towards human HepG2 cells. The two selected candidates ($AntiOxCIN_4$ and $AntiOxCIN_6$) prevented iron- and hydrogen peroxide-induced cytotoxicity without disturbing mitochondrial morphology and polarization. Both $AntiOxCIN_4$ and $AntiOxCIN_6$ altered the redox state of the primary human skin fibroblasts cells and produced a mild increase in reactive oxygen species (ROS) production and oxidative stress *in situ*, which triggered an up-regulation of antioxidant defenses. Notwithstanding, the mild increase in intracellular ROS levels was not associated with alterations in mitochondrial morphology and function and/or with cell death. Moreover, $AntiOxCIN_4$ increased GSH levels and MnSOD protein concentration. Thus, it is likely that $AntiOxCIN_4$ up-regulate the intracellular antioxidant defense system as a result of an adaptative response of cells, a process that can protect them against subsequent stress-inducing events.

In summary, new mitochondriotropic antioxidants based on dietary scaffolds were successfuly developed. This new systems can be applied in a next future as first-class drugs for the treatment of mitochondrial oxidative stress related diseases.

Acknowledgments: 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), (Pest-C/SAU/LA0001/2013-2014) and (PTDC/DTP-FTO/2433/2014). J Teixeira (PTDC/DTP-FTO/2433/2014), F. Cagide (SFRH/BPD/74491/2010) and S. Benfeito (SFRH/BD/99189/2013) grants are supported by FCT, POPH and QREN.

Poster communications

Poster communication 1 - Working Group 1

Unravelling triterpene-hydroxycinnamate biosynthesis in apple

Christelle M Andre,[a] Sylvain Legay,[a] Jean-Francois Hausman[a]

[a] *Environmental Research and Innovation Department, Luxembourg Institute of Science and Technology, L-4362 Esch/Alzette, Luxembourg*

christelle.andre@list.lu

Pentacyclic triterpenes possess numerous biological properties, including anti-inflammatory, anti-cancer, and anti-plasmodial activities. Ursolic and oleanolic acids, of the ursane and oleanane triterpene types, respectively, predominate in the skins of most commercial apple varieties [1]. In contrast, we showed that russeted old heritage apple cultivars, characterized by the accumulation of suberin in skin tissues [2,3], have higher concentrations of lupane derivatives, including betulinic acid and specific conjugated triterpenes such as betulinic acid-3-trans-caffeate, as compared to their waxy-skinned counterparts [4].

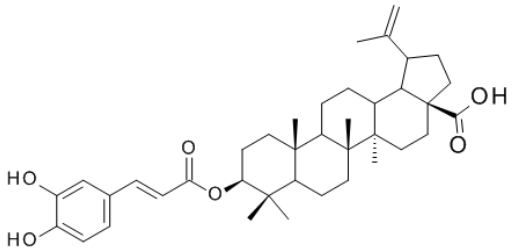


Figure 1: Structure of a Russet apple-specific triterpene-hydroxycinnamate: betulinic acid-3-trans-caffeate.

Triterpene esters, such as triterpene-hydroxycinnamates are of particular interest for the pharmaceutical industry as they have been reported with increased anti-inflammatory, anti-malarial and anti-cancer activities as compared to non-conjugated triterpenes. In a recent metabolomics study on two nearly isogenic yet contrasting varieties, *i.e.* 'Canada Gris' (russeted skin) and 'Canada Blanc' (waxy skin), triterpene-coumarates were also identified in waxy tissues. Previous studies performed by our group on triterpene biosynthesis in apple have highlighted the involvement of two key multifunctional oxidosqualene cyclases (MdOSC1, MdOSC5) and one cytochrome P450 (CYP716A175) in the production of the triterpenic acids [5]. Our current efforts aim at identifying hydroxycinnamoyl-CoA transferases responsible for the final esterification step. Taken together, these results will provide important information for the large scale *in vitro* production of specific apple triterpenes via bioengineering approaches.

References:

[1] Andre CM, Greenwood J, Walker E, Rassam M, Sullivan M, Evers D, Perry N, Laing W. Anti-inflammatory procyanidins and triterpenes in 109 apple varieties. *Journal of Agricultural and Food Chemistry*, **2012**, *60*: 10546–10554.

[2] Legay S, Guerriero G, Deleruelle A, Lateur M, Evers D, Andre CM, Hausman JF. Apple russeting as seen through the RNA-seq lens: strong alterations in the exocarp cell wall. *Plant Molecular Biology*, **2015**; *88*: 21-40.

[3] Legay S, Guerriero G, Andre CM, Guignard C, Cocco E, Charton S, Boutry M, Rowland O, and Hausman JF. MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. *New Phytologist*, *In press*, **2016**.

[4] Andre CM, Larsen L, Burgess EL, Jensen DJ, Cooney JM, Evers D, Zhang J, Perry NB, Laing WA. Unusual immuno-modulatory triterpene caffeates in the skins of russeted varieties of apples and pears. *Journal of Agricultural and Food Chemistry*, **2013**; *61*: 2773-2779.

[5] Andre CM, Legay S, Deleruelle A, Lateur M, Nieuwenhuizen N, Punter M, Brendolise C, Cooney J, Lateur M, Hausman JF, Larondelle Y, Laing Y. Multifunctional oxidosqualene cyclases and cytochrome P450 involved in the biosynthesis of apple fruit triterpenic acids. *New Phytologist*, **2016**, *211*: 1279–1294.

Poster communication 2 - Working Group 1

Discovery of novel gyrase B and gyrase B/topoisomerase IV (ParE) dual inhibitors with in vitro antibacterial activity

Michaela Barančoková,[a] Janez Ilaš,[a] Tihomir Tomašič,[a] Päivi Tammela,[b] Danijel Kikelj[a]

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

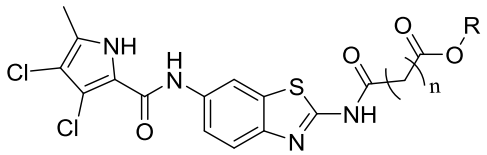
[b] *Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland*

michaela.barancokova@ffa.uni-lj.si

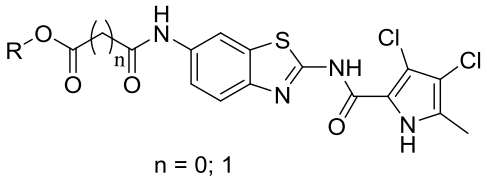
DNA gyrase and topoisomerase IV belong to topoisomerase IIA class of enzymes. They have crucial role for the cells, as they are involved in catalysis of interconversion of topological state of DNA and thus enable processes such as replication or chromosome. By cleaving both strands of DNA they perform negative supercoiling behind replication fork (unique action of DNA gyrase) and decatenation of daughter DNA strand after replication (topoisomerase IV). Nowadays, with increasing threat of bacterial resistance, those enzymes are attractive targets for antibacterial drug discovery. Both gyrase B and Topo IV are heterotetramers composed of two subunits (A_2B_2, C_2E_2). We focused on discovery of inhibitors of GyrB and ParE subunits targeting their ATP binding site as a design principle.

We prepared new 3,4-dichloro-5-methylpyrrole-2-carboxamide analogues of gyrase inhibitors recently synthesized and published by our group[1]. We replaced dibromopyrrole-/dichloropyrrolecarboxamide moiety attached to position 2 or 6, respectively, of benzo[*d*]thiazole-2,6-diamine scaffold with 3,4-dichloro-5-methylpyrrole-2-carboxamide group to complete structure-activity relationship in the series and obtained improved analogues with promising inhibitory activity against both gyrase B and topoisomerase IV.

Efforts to improve physico-chemical properties of synthesized compounds which would enable their in vitro antibacterial activity (e.g. by replacing the benzothiazole scaffold with benz[*d*]imidazole scaffold) are in progress.



n = 0; 1
R = Me; Et; H



n = 0; 1
R = Me; Et; H

References:

[1] Gjorgjieva, M.; Tomašič, T.; Barančokova, M.; Katsamakas, S.; Ilaš, J.; Tammela, P.; Peterlin Mašič, L.; Kikelj, D. Discovery of Benzothiazole Scaffold-Based DNA Gyrase B Inhibitors. *J. Med. Chem*, **2016**, *59*, 8941−8954.

Acknowledgements: This work was supported by Marie Curie Skłodowska Curie ETN INTEGRATE (Contract 642620).

Poster communication 3 - Working Group 1

Development of bifunctional agents for ameliorating the oxidative stress associated with aging related diseases

Sofia Benfeito,[a,b] Fernando Remião,[b] Eugenio Uriarte,[c] Fernanda Borges[a]

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

[b] *Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal*

[c] *Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, Spain*

Sofia_benfeito@hotmail.com

Aging-related neurodegenerative diseases, such as Alzheimer's disease (AD), are pathologies characterized by a progressive and irreversible neuronal death and a central nervous system dysfunction. As AD is a multifactorial disease, the development of therapeutic agents that can reach two or more pharmacological targets involved in the neurodegenerative pathological cascade is attracting progressively more attention. The generation of intracellular reactive species (ROS and RNS) by mitochondria dysfunction is thought to be one of the mechanisms that can lead to the progressive and irreversible neuronal death. In this context, it is believed that the modulation of mitochondrial function by exogenous antioxidants can be a strategy to prevent or delay the deleterious oxidative stress effects in neurodegenerative diseases. However, despite their interesting *in vitro* antioxidant profile its application in therapy was not successful. Failure of therapy is often associated with restrains related with physicochemical properties, particularly low lipophilicity and bioavailability.

The main goal of this project is the development of innovative centrally active mitochondriotropic hydroxycinnamic derivatives with neuroprotective activity and able to cross he blood-brain barrier (BBB). The scaffold is a naturally occurring hydroxycinnamic acid (HCA) that has frequently been used as a model for the design and development of new antioxidants. Structural characterization of the newly synthesized compounds was carried out by NMR spectroscopy (^{1}H, ^{13}C and DEPT) and electronic impact mass spectroscopy (MS/IE). Biological screening has included the assessment of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, *in vitro* antioxidant activity, *in vitro* blood-brain barrier permeation ability and the evaluation of compound´s cytotoxicity in SH-SY5Y neuroblastoma cell lines. Mitochondrial functional assays are in progress. 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/00081/2015, POCI-01-0145-FEDER-006980, NORTE-01-0145-FEDER-000028 and **PTDC/DTP-FTO/2433/2014)**.

S. Benfeito thanks the Foundation for Science and Technology (FCT) doctoral grant (SFRH/BD/99189/2013).

Poster communication 4 - Working Group 1

Development of a new class of benzensulfonamides as selective CA inhibitors

Elvira Bruno,[a] Maria Rosa Buemi,[a] Laura De Luca,[a] Stefania Ferro,[a] Claudiu T. Supuran,[b] Andrea Angeli,[b] Roberto Cirilli,[c] Daniele Sadutto,[c] Anna Di Fiore,[d] Vincenzo Alterio,[d] Giuseppina De Simone,[d] Rosaria Gitto[a]

[a]*Department of CHIBIOFARAM, University of Messina, Viale Annunziata, I-98168, Messina, Italy.*

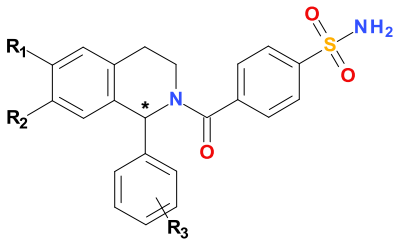
[b]*Department of NEUROFARBA, University of Florence, Via Ugo Schiff 6, I-50019 - Sesto Fiorentino, Italy.*

[c]*Department of Farmaco, Istituto Superiore di Sanità, V.le Regina Elena, 299, 00161 Rome, Italy.*

[d]*Institute of Biostrutture e Bioimmagini-CNR, Via Mezzocannone 16, 80134, Naples, Italy.*

brunoe@unime.it

Carbonic anhydrases (CA) are metalloenzymes that catalyse the reversible hydration of carbonic dioxide to bicarbonate ion and proton. This family of enzyme comprises 15 different α- isoforms which are widely distributed in different tissues and organs. The abnormal levels or activities of these enzymes have been associated with human diseases such as cancer, epilepsy, obesity, etc.[1] Specifically, the hCA VII, hCA IX and hCA XIV isoforms have shown to be amenable druggable targets.[2] As an extension of previous studies,[3] we focused our interest on the class of 1-aryl-1,2,3,4-tetrahydroisoquinoline derivatives bearing the benzensulfonamide moiety as a metal binding group, which is considered the structural requirement for CA inhibition. Some of these new synthesized sulfonamides demonstrated relevant CA inhibitory activity especially toward hCA VII (K_i < 85 nM), which is highly distributed in CNS. Moreover, we studied the role of stereochemistry during the binding process into the CA binding site thus carrying out the enantiomeric resolution of selected compounds. Finally, to explore the mechanism of enzyme inhibition and the placement in the catalytic pocket, we performed structural studies by means of experimental and computational approaches.



References:

[1] Gitto, R.; Agnello, S.; Ferro, S.; Vullo, D.; Supuran, C. T.; Chimirri, A. Identification of Potent and Selective Human Carbonic Anhydrase VII (hCA VII) Inhibitors. *ChemMedChem*, **2010**, *5*, 823-826.

[2] Gitto, R.; Damiano, F. M.; Mader, P.; De Luca, L.; Ferro, S.; Supuran, C. T.; Vullo, D.; Brynda, J.; Řezáčová, P.; Chimirri, A. Synthesis, Structure−Activity Relationship Studies, and X-ray Crystallographic Analysis of Arylsulfonamides as Potent Carbonic Anhydrase Inhibitors. *J. Med. Chem*, **2012**, *55*, 3891−3899.

[3] Buemi, M. R.; De Luca, L.; Ferro, S.; Bruno, E.; Ceruso, M.; Supuran, C. T.; Pospíšilová, K.; Brynda, J.; Řezáčová, P.; Gitto, R. Carbonic anhydrase inhibitors: Design, synthesis and structural characterization of new heteroaryl-N-carbonylbenzenesulfonamides targeting druggable human carbonic anhydrase isoforms. *Eur. J. Med. Chem*, **2015**, *102*, 223-232.

This work was supported by University of Messina Research & Mobility 2015 Project (Project Code: RES_AND_MOB_2015_DE_LUCA).

Poster communication 5 - Working Group 1

1-Substituted tetrahydroisoquinolines and 7-phenyl-hexahydrocyclopenta[ij] isoquinolines as D2-like dopaminergic receptor ligands

Nuria Cabedo,[a] Javier Párraga,[b] N. El Aouad,[c] María Jesús Sanz,[d] Ricardo D. Enriz,[e] Diego Cortes[b]

[a] *Health Research Institute of INCLIVA, Menéndez Pelayo 4, 46010 Valencia, Spain*

[b] *Dept of Pharmacology, Faculty of Pharmacy of University of Valencia, Vicent Andres Estellés s/n, 46100 Valencia, Spain*

[c] *Campus Universitaire Ait Melloul, Universit_e Ibn-Zohr, Agadir, Morocco*

[d] *Dept of Pharmacology, Faculty of Medicine of University of Valencia, Blasco Ibañez 15, 46010 Valencia, Spain*

[e] *Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis-IMIBIO-SL, Chacabuco 915, 5700, San Luis, Argentina*

ncabedo@uv.es

Imbalances in dopamine neurotransmission provoke the apparition of neurological and psychiatric disorders, including Parkinson, schizophrenia and depression that affect to 10 million, 21 million and 121 million people worldwide, respectively. Three series of dopamine receptor (DR) ligands containing tetrahydroisoquinoline (THIQ) nucleus were synthetized and tested *in vitro* for their affinity to D_1-like and D_2-like DR in rat striatum: (*E*)-1-styryl-THIQs (series 1), 7-phenyl-hexahydrocyclopenta[*ij*]-IQs (HCPIQs) (series 2) and (*E*)-1-(prop-1-en-1-yl)-THIQs (series 3).[1,2]

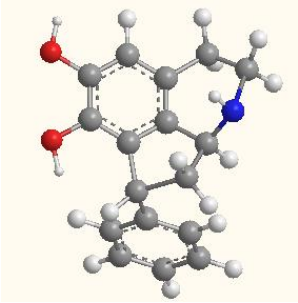


Figure 1: 7-Phenyl- 1,2,3,7,8,8a-hexahydrocyclopenta[*ij*] isoquinoline **3a**.

We observed that both the catechol group and *N*-substitution were determinant motifs to bind the D_2-DR. In addition, the introduction of a cyclopentane ring led to HCPIQ compounds provided of high affinity but also an unexpected selectivity to D_2-DR, with Ki D_1/D_2 ratio values of 2465, 1010 and 382 for compounds **3a**, **3c** and **3e**, respectively.[2] This D_2-DR binding selectivity was supported by molecular modeling studies. None of the most active THIQs in D_2 DR displayed relevant cytotoxicity in human neutrophils and HUVEC.

References:

[1] Párraga, J.; Galán, A.; Sanz, M.J.; Cabedo, N.; Cortes, D. Synthesis of hexahydrocyclopenta[ij]isoquinolines as a new class of dopaminergic agents. *Eur. J. Med. Chem*. **2015**, *90* 101-106.

[2] Párraga, J.; Andújar, S.; Rojas, S.; Gutierrez, L.J.; El Aouad, N.; Sanz, M.J.; Enriz, D.R.; Cabedo, N.; Cortes, D. Dopaminergic isoquinolines with hexahydrocyclopenta[*ij*]-isoquinolines as D_2-like selective ligands. *Eur. J. Med. Chem*. **2016**, *122*, 27-42.

Poster communication 6 - Working Group 1

Design and development of novel and selective A3 adenosine receptor ligands based on chromone-2-carboxamide

Fernando Cagide,[a] Joana Reis,[a] Daniel Chavarria,[a] Santiago Vilar,[b,c] George Hripcsak,[c] Eugenio Uriarte,[b] Sonja Kachler,[d] Karl-Norbert Klotz[d] and Fernanda Borges[a]

[a] CIQUP/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal.
[b] Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain.
[c] Department of Biomedical Informatics, Columbia University Medical Center, 10032, New York, USA
[d] Institut fur Pharmakologie und Toxikologie, Universitat Wurzburg, 97078 Wurzburg, Germany

fernandocagide@yahoo.es

Adenosine is a purine nucleoside that acts as a signaling molecule through the activation of four G-protein-coupled adenosine receptor (AR) subtypes denoted as A_1, A_{2A}, A_{2B} and A_3.[1] These receptors are considered to be attractive drug targets as they are widely expressed and implicated in several physiological and pathological biological functions. However, one of the major hurdles in the development of safe and effective drugs targeting G-protein coupled receptors (GPCRs) is finding ligands that are selective for a specific receptor subtype. The increasing knowledge about the biological, physiological and pathological role of the AR subtypes has been accompanied by a number of projects focused on the design and development of the AR ligands. Nevertheless, the development and role of A_3AR agonists and antagonists is still an open issue.[2]

Preceding results attained by our group reveal that chromone-2-phenylcarboxamide and its derivatives have a remarkable preference for the hA$_3$AR. As a result, additional structure–affinity-relationship (SAR) studies have been accomplished and a chromone-2-thiazolecarboxamide derivative has emerged as the most potent and selective ligand (hA$_3$ *Ki* of 167 nM and a selectivity ratio of 590 vs. the hA$_1$ and 480 vs. the hA$_{2A}$AR subtypes).[3] Subsequent studies have been performed in order to improve ligands solubility by introducing particular structural modifications; thus attaining ligands with improved drug-like properties but still maintaining the hA$_3$ *Ki* nanomolar activity. The work herein described was also supported by molecular docking studies to understand the putative interactions and the selectivity of the compounds to hA$_3$AR relatively to hA$_1$ hA$_{2A}$AR.

References:

[1] Chen, J. F., Eltzschig, H. K and Fredholm B. B., *Nat. Rev. Drug Discovery*, **2013**, 12, 265–286.

[2] Borea P. A., Varani K., Vincenzi F., Baraldi P. G., Tabrizi, M. A. Merighi S. and Gessi S., *Pharmacol. Rev*., **2015**, *67*, 74–102.

[3] Cagide F., Gaspar A., Reis J., Chavarria D., Vilar S., Hripcsak G., Uriarte E., Kachler S., Klotz K. N. and Borges F., *RSC Adv.*, **2015**, *5*, 78572–78585.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980). J.Reis (SFRH/BD/96033/2013) and F Cagide ((SFRH/BPD/74491/2010) were also supported by FCT and FEDER/COMPETE funds.

Poster communication 7 - Working Group 1

Total synthesis of ent-smenamide A and C-16-epi-smenamide A

Alessia Caso,[a] Vincenzo Piccialli,[b] Alfonso Mangoni,[a] Giorgia Oliviero,[a] Gennaro Piccialli,[a] Valeria Costantino[a]

[a]*Department of Pharmacy, University of Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy*

[b]*Department of Chemical Sciences, University of Napoli Federico II, via Cynthia 4, I- 80126 Napoli, Italy*

alessia.caso@unina.it

Smenamides A is a cytotoxic chlorinated hybrid peptide/polyketide isolated from the Caribbean sponge *Smenospongia aurea*[1]. Smenamide A is present only in microgram amounts in the natural source. Therefore, its total synthesis was undertaken to obtain larger amounts of the compound for an in-depth study of its biological activity and to allow the elucidation of the configuration at C16 as well as to provide analogs of smenamide A for SAR studies.

From a retrosynthetic point of view (figure 1), the molecule was disconnected in two building blocks, fragments A and B. In particular, the central part of smenamide A was easily related to citronellene. However, the enantiomeric identity of the starting citronellene was an arbitrary choice. Thus, starting from *S*-citronellene, fragment A has been constructed. Its coupling with the dolapyrrolidone unit (fragment B), prepared from L-Phe as described[2], completed the synthesis.

The comparison between the ^{1}H NMR spectrum of the synthetic material (**1**) and that of the natural product showed that we had synthesized the C16-*epi*-smenamide A and that the natural substance had to possess an R configuration at C-16. In order to confirm this deduction the enantiomer of fragment B was prepared starting from D-Phe and coupled with fragment A affording *ent*-smenamide A (**2**). Its proton spectrum was in perfect agreement with that of the natural compound. Finally, with the optimized synthetic route at hand, the synthesis of smenamide A will be easily carried out, from *R*-citronellene.

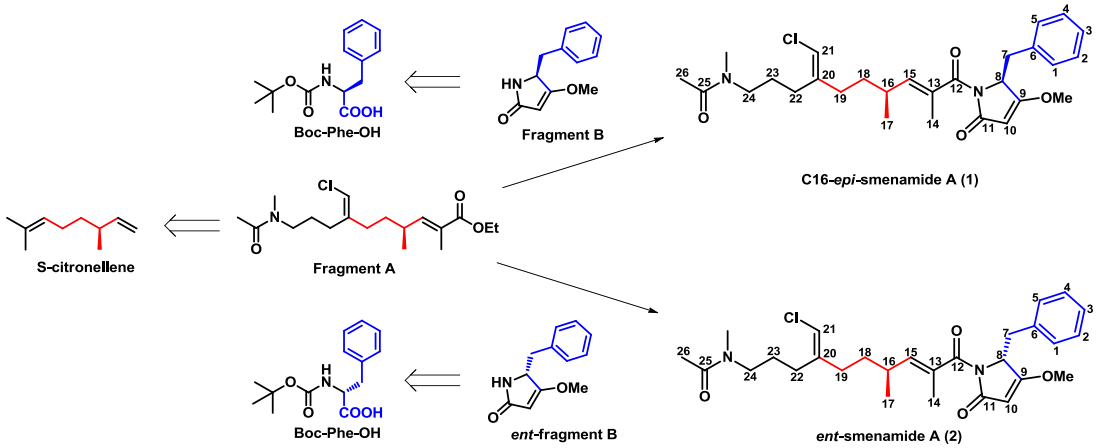


Figure 1: retrosynthetic analysis.

References:

[1] Teta R.; Irollo E.; Della Sala G.; Pirozzi G.; Mangoni A.; Costantino V., *Mar. Drugs*, **2013**, 11, 4451-4463.

[2] Conroy T.; Guo J.T.; Linington R.G.; Hunt N.H.; Payne R.J., *Chem. Eur. J.* **2011**, *17*, 13544-13552

Poster communication 8 - Working Group 1

Insights on the development of novel neuroprotective agents: a comparative study between ferulic acid and related sulfanyl derivatives

Daniel Chavarria,[a] Tiago Silva,[a] Jorge Garrido,[b] Fernando Remião[c] and Fernanda Borges[a]

[a] *CIQ/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.*
[b] *Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic of Porto, 4200-072 Porto, Portugal.*
[c] *UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*

danielfchavarria@gmail.com

Polyphenols represent a structurally heterogeneous class of compounds endowed with multiple biological properties. Their antioxidant activity may counterbalance the disproportionate production of reactive species in oxidative stress-related disorders such as neurodegenerative diseases.[1] Over the last decade, several modifications of the polyphenols backbone have been performed to obtain new antioxidants with higher efficiency and potency. These approaches can include, for instance, the replacement of the hydroxyl group (OH) by a sulfhydryl group (SH)[2] and the introduction of lipophilic substituents.[3]

Ferulic acid (FA) is a well-known antioxidant with strong cytoprotective activity.[4] However, its unfavorable physicochemical properties reduce its bioavailability and restrict the access to pharmacological targets.[4] The goal of the present work is the design and development of new FA derivatives (Figure 1) endowed with neuroprotective activity.

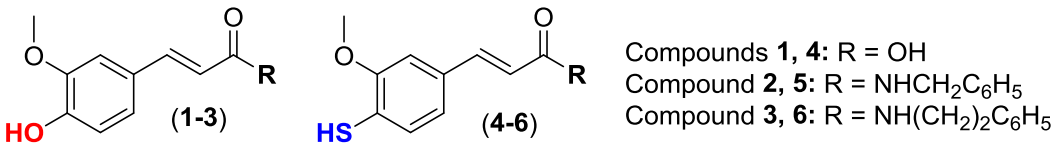


Figure 1: Chemical structure of FA (1) and related derivatives (2-6).

FA-based antioxidants were successfully obtained. The antioxidant activity of the synthesized compounds was assessed by total antioxidant capacity assays, namely DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and galvinoxyl methods, and the evaluation of the redox potential by differential pulse voltammetry. Cell-based assays were performed in SH-SY5Y neuroblastoma cell lines in order to evaluate the cytotoxicity of the compounds as well as the neuroprotection profile against 6-hydroxydopamine (6-OHDA)-induced damage. 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). f D. Chavarria (SFRH/BD/108119/2015) grant was also supported by FCT and FEDER/COMPETE funds.

References:

1. Benfeito, S. *et al.* Antioxidant therapy: still in search of the 'magic bullet'. *Mitochondrion* **2013**, *13,* 427–435.

2. Cao, X. Y. *et al.* Extraordinary radical scavengers: 4-mercaptostilbenes. *Chem. - A Eur. J.* **2012,** *18,* 5898–5905.

3. Teixeira, J. *et al.* Exploring nature profits: development of novel and potent lipophilic antioxidants based on galloyl-cinnamic hybrids. *Eur. J. Med. Chem.* **2013,** *62,* 289–296.

4. Mancuso, C. & Santangelo, R. Ferulic acid : Pharmacological and toxicological aspects. *Food Chem. Toxicol.* **2014**, *65,* 185–195.

Poster communication 9 - Working Group 1

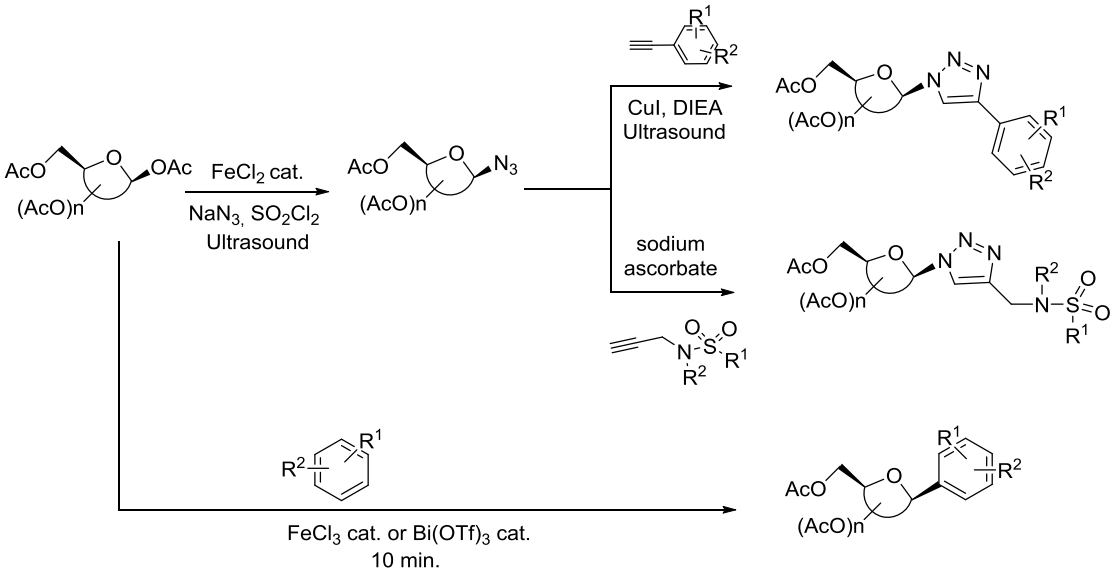
A chemical toolbox applied to original syntheses of druggable C-nucleosides analogues

Luc Demange,[a,b] Soukaina Alaoui,[b,c] Hamza Tachallait,[b,c] Aziza Saber,[b,c] Hamid Marzag,[b,c] Khalid Bougrin[c] and Rachid Benhida[b]

[a] Université Paris-Descartes, Sorbonne Paris Cité, UFR des Sciences Pharmaceutiques et UFR Biomédicale des Saints Pères, Paris, France.
[b] ICN, UMR CNRS 7272, Université de Nice Sophia-Antipolis-Université Côte d'Azur , Parc Valrose, Nice, France.
[c] Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, URAC23, Faculté des Sciences, Université Mohammed V, B.P. 1014 Rabat, Maroc.

lucdemange@yahoo.ca

C-nucleosides are widely used in medicinal chemistry due to their high value as therapeutic agents and biochemical probes. For example, tiazofurin has been approved as an orphan drug for the treatment of certain type of haematological malignancies. Various approaches have been used for the synthesis of C-nucleosides. We report here two routes which have been developed by our team. The first one, consists in the use of a Friedel-Craft mediated by an Lewis acid as catalysts, which leads to aromatic ribosides and glucosides in high yields and with an excellent stereoselectivity. The second route is based on the use of azaglycosides as substrates for a subsequent functionalization through click-chemistry. Among the series of C-nucleosides synthesized in the team, some products revealed an interesting cytotoxic activity against haematological malignancies.



References

[1].Spadafora, M.; Mehiri, M.; Burger, A. and Benhida, R. *Tetrahedron Lett.*, 2008, **49**, 3967.

[2].Driowya, M.; Puissant, A.; Robert, G.; Auberger, P. Benhida, R., and Bougrin, K. *Ultrason. Sonochem.*, 2012, **19**, 1132.

[3]. Marzag, H. ; Alaoui, S. ; Amdouni, H. ; Martin, A.R. ; Bougrin, K., and Benhida, R. *New J. Chem.*, 2015, **39**, 5437.

Poster communication 10 - Working Group 1

Design and synthesis of potent anti-influenza small molecules that disrupt the RNA polymerase PA-PB1 subunits interaction

Jenny Desantis,[a] Serena Massari,[a] Giulio Nannetti,[b] Laura Goracci,[c] Arianna Loregian,[b] Oriana Tabarrini[a]

[a] *Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, Perugia, Italy*

[b] *Department of Molecular Medicine, University of Padua, Via A. Gabelli 63, Padua, Italy*

[c] Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di sotto 8, Perugia, Italy

jenny.desantis@hotmail.it

The limited therapeutic options against the influenza virus (Flu) infection together with the rapid emergence of drug resistance strains make imperative the search for next-generation agents. In this context, the Flu RNA-dependent RNA polymerase (RdRp) and in particular its correct assembly to a heterotrimer composed by PA, PB1, and PB2 subunits, has been elected as an attractive target for a challenging but strategic protein-protein interaction (PPI) inhibition approach.[1] The inhibition of a viral polymerase is a commonly used approach for the identification of antiviral agents, but the development of PPI inhibitors able to interfere with viral polymerase assembly is an innovative strategy that may have major advantages, of which the most important is a probable decrease in drug resistance emergence.

During the last four years, the inhibition of the Flu RdRp PA-PB1 subunits interface has become an active field of research, following the publication of PA-PB1 crystal structures.[1] Our research group has identified many of the small molecule PA-PB1 complex formation inhibitors reported to date, thanks to an initial Structure-Based Virtual Screening that led to identify five hit compounds,[2] followed by their optimization.[3-5] A first hit-to-lead optimization phase performed on one of these hits, the cycloheptathiophene-3-carboxamide derivative **10**, and focused on mainly modifying the moiety placed at the C-2 position of the scaffold, led to an increased PA-PB1 interaction inhibition and a more potent anti-Flu activity also encompassing clinical isolates and drug-resistant strains.[3] In order to improve the biological activity of this class of compounds and acquire further SAR information on the other moieties of the scaffold, a second hit optimization phase was performed. From the biological evaluation of the whole set of compounds, new derivatives endowed with an interesting ability to interfere with the PA-PB1 heterodimerization that well translated to good Flu replication inhibition have been identified. In this work, the design, synthesis, biological evaluation, and hypothesized binding mode of this new series of cycloheptathiophene-3-carboxamide compounds, will be presented.

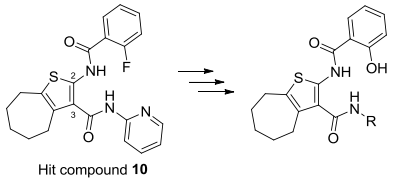


Figure 1: Cycloheptathiophene-3-carboxamide scaffold evolution

References:

[1] Massari, S.; Goracci, L.; Desantis, J; Tabarrini, O. Polymerase acidic protein-basic protein 1 (PA-PB1) protein-protein interaction as a target for next-generation anti-influenza therapeutics.. *J. Med. Chem.*, **2016**, *59*, 7699-7718.

[2] Muratore, G.; Goracci, L.; Mercorelli, B.; Foeglein, Á.; Digard, P.; Cruciani, G.; Palù, G.; Loregian, A. Small molecule inhibitors of influenza A and B viruses that act by disrupting subunit interactions of the viral polymerase. *Proc. Natl. Acad. Sci. USA*, **2012**, *109*, 6247-6252.

[3] Massari, S.; Nannetti, G.; Goracci, L.; Sancineto, L.; Muratore, G.; Sabatini, S.; Manfroni, G.; Mercorelli, B.; Cecchetti, V.; Facchini, M.; Palù, G.; Cruciani, G.; Loregian, A.; Tabarrini, O. Structural investigation of cycloheptathiophene-3-carboxamide derivatives targeting influenza virus polymerase assembly. *J. Med. Chem.*, **2013**, *56*, 10118-10131.

[4] Lepri, S.; Nannetti, G.; Muratore, G.; Cruciani, G.; Ruzziconi, R.; Mercorelli, B.; Palù, G.; Loregian, A.; Goracci, L. Optimization of small-molecule inhibitors of influenza virus polymerase: from thiophene-3-carboxamide to polyamido scaffolds. *J. Med. Chem.*, **2014**, *57*, 4337-4350.
[5] Massari, S.; Nannetti, G.; Desantis, J.; Muratore, G.; Sabatini, S.; Manfroni, G.; Mercorelli, B.; Cecchetti, V.; Palù, G.; Cruciani, G.; Loregian, A.; Goracci, L.; Tabarrini, O. A Broad anti-influenza hybrid small molecule that potently disrupts the interaction of polymerase acidic protein-basic protein 1 (PA-PB1) subunits. *J. Med. Chem.*, **2015**, *58*, 3830-3842.

Poster communication 11 - Working Group 1

Homobivalent carbolines as potential designed multiple ligands for the therapy of neurodegenerative disorders

Christoph Enzensperger,[a] Robert Penzis,[b] Doreen Bönisch,[b] Thomas Winckler[b]

[a] *Dept of Organ. and Macromoleculare Chemistry, Friedrich Schiller University, 07745 Jena, Germany*

[b] *Dept of Pharmaceutical Biology, Institute of Pharmacy, Friedrich Schiller University, 07745 Jena, Germany*

ch.enzensperger@uni-jena.de

Neurodegenerative diseases represent a challenge for biomedical research due to their high prevalence and lack of mechanism-based treatments. Because of the complex pathology of neurodegenerative disorders, multi-functional drugs have been increasingly recognized as potential treatments. We identified homobivalent γ-carbolinium salts as potent inihitors of both cholinesterases, *N*-methyl-D-aspartate (NMDA) receptors, and monoamine oxidases. Homobivalent γ-carbolines displayed similar structure-activity relationships and required 7-9 carbon linkers and quaternary pyrido nitrogens to act as inhibitors on the tested targets. The most potent compound RO-44 (Figure 1) displayed nanomolar inhibitory potency at acetyl- and butyrylcholinesterase with a 35-fold specificity for the former. On GluN1-1/GluN2A and GluN1-1/GluN2B NMDA receptors, RO-44 displayed submicromolar inhibitory activity in a cell-based assay of glutamate-induced cytotoxicity with IC_{50} values tenfold below the approved anti-Alzheimer drug memantine. In addition, RO-44 emerged as a subnanomolar non-selective inhibitor of monoamine oxidases MAO-A and MAO-B. RO-44 displays low general cytotoxicity on cell culture cells and preliminary data from an in vitro blood brain barrier model suggest that the compound may be able to reach the CNS. Thus, RO-44 is a promising designed multiple ligand for the combination therapy of neurodegenerative disorders.

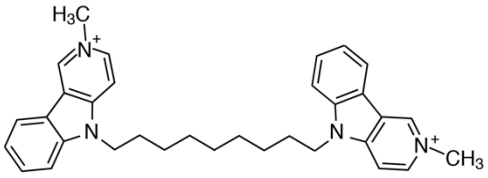


Figure 1: Structure of the homobivalent γ-carbolinium RO-44.

References:

[1] Rook, Y.; Schmidtke, K.-U.;Gaube, F.; Schepmann, D.; Wünsch, B.; Heilmann; J.;Lehmann, J.; Winckler, T. Bivlant γ-carbolines as potential multi-target anti-Alzheimer agents. *J. Med. Chem.*, **2010**, *53*, 3611-3617.

[2] Otto, R.; Penzis, R.; Gaube, F.; Adolph, O.; Föhr, K.J.; Warncke, P.; Appenroth, D.; Fleck, C.; Enzensperger, C.; Lehmann, J.; Winckler, T. Evaluation of homobivalent carbolines as designed multiple ligands for the treatment of neurodegenerative disorders. *J. Med. Chem.*, **2015**, *58*, 6710-6715.

Poster communication 12 - Working Group 1

Nanomedicine as a tool to surpass physicochemical and bioavailability constrains: delivering of a coumarin-based MAO-B inhibitor using functionalized PLGA nanoparticles

Carlos Fernandes,[a] André Fonseca,[a] Rute Nunes,[b,c,d] Bruno Sarmento,[b,c,d,e] Fernando Remião,[f] Fernanda Borges[a]

[a] *CIQUP/Departament of Chemistry, Faculty of Sciences, University of Porto, Porto, 4169-007, Portugal*
[b] *i3S, Institute of Research and Inovation in Health, Porto, 4200-135, Portugal,*
[c] *INEB, Institute of Biomedical Engineering, Biocarrier Group, Porto, 4200-135, Portugal,*
[d] *ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, 4050-313, Portugal*
[e] *CESPU, Gandra, 4585-116, Portugal*
[f] *UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, 4050-313, Portugal*

cevinhas@gmail.com

Central nervous system (CNS) disorders, such as Alzheimer or Parkinson's diseases, are among the most emergent, disabling and yet non curable diseases of the 21th century. These diseases are characterized as unsolved pathologies as their etiologies are not completely understood. Furthermore, the surpassing of potential therapeutic or diagnostic compounds through protective biological barriers, such as blood-brain barrier (BBB), remains a challenge as it limits the access to CNS.

Recent developments in nanoscience and nanotechnology have given rise to a new generation of functional nanomaterials with controlled morphology and well-defined properties that can be part of a solution to solve several drawbacks of drug discovery and development programs. In particular, poly-(lactic-co-glycolic acid) nanoparticles (PLGA NPs) have been widely reported as promising carriers that can stabilize lipophilic or hydrophilic compounds enhancing for instance their bioavailability, namely half-life blood circulation and accumulation in specific targets or tissues[1].

Moreover, the presence of polyethylene glycol (PEG) in NPs surface had been reported as a method to improve their stealth properties, increasing as well their ability to cross BBB[2].

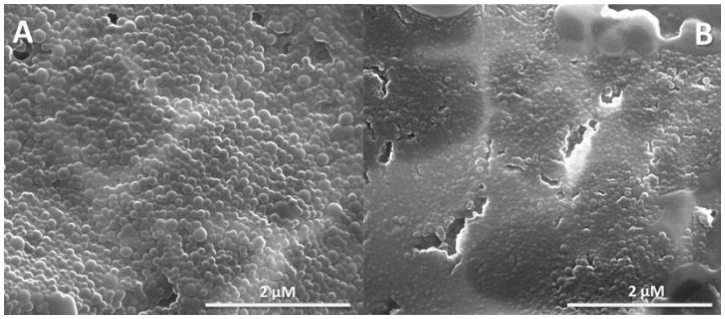


Figure 1: SEM images of PEGylated coated PLGA NPs without (A) and with coumarin encapsulated (B).

In this context, the aim of our project was to develop a brain-targeting nanoformulation to solve the solubility problems of a potent and selective MAO-B inhibitor based on coumarin scaffold[3]. PLGA nanoparticles (Figure 1) were prepared by nanoprecipitation method and the functionalization of their surface was performed using a PEGylated surfactant – Kolliphor 188. The morphological physicochemical and biological properties of the novel formulations will be presented in this communication.

References:

[1] Jose, S., Sowmya, S., Cinu, T.A., Aleykutty, N.A., Thomas, S., Souto, E.B., Surface modified PLGA nanoparticles for brain targeting of Bacoside-A, *Eur. J. Pharm. Sci.*, **2014,** 63, 29-35.

[2] Nance, E.A., Woodworth, G.F., Sailor, K.A., Shih, T.T.-Y., Swaminathan, G., Xiang, D., A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue, *Sci. Transl. Med.*, **2013**, 4, 119-149.

[3] Matos, M. J., Vilar, S., Gonzalez-Franco, R. M., Uriarte, E., Santana, L., Friedman, C., Tatonetti, N. P., Viña, D., Fontenla, J. A., Novel (coumarin-3-yl)carbamates as selective MAO-B inhibitors: synthesis, in vitro and in vivo assays, theoretical evaluation of ADME properties and docking study, *Eur. J. Med. Chem*., **2013**, 63, 151-161.

Poster communication 13 - Working Group 1

Coumarin versus chromone monoamine oxidase B inhibitors: Quo vadis?

André Fonseca,[a,b] Joana Reis,[a] Maria Matos,[a,b] Donatella Bagetta,[c] Francesco Ortuso,[c] Stefano Alcaro,[c] Eugenio Uriarte,[b] Fernanda Borges[a]

[a] *CIQUP/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal.*

[b] *Departamento de Química Orgánica, Faculdade de Farmacia, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Espanha*

[c] *Dipartimento di Scienze della Salute, Università degli Studi "Magna Græcia" di Catanzaro, Campus "Salvatore Venuta", Viale Europa, 88100, Catanzaro, Italy*

andrefonsecaa12@gmail.com

In modern society there is a rise of diagnosed cases of neurodegenerative diseases (ND), mainly Parkinson (PD) and Alzheimer (AD) diseases. Neither of these diseases has an effective treatment, and the drugs currently available are only used to delay the progress of neurodegeneration by controlling its symptoms. Monoamine oxidases (MAOs) are enzymes present in the outer mitochondrial membrane of mammalian cells with two known isoforms, MAO-A and MAO-B. They catalyze the oxidation of diverse biogenic amines such as dopamine, which is an important neurotransmitter linked to PD. Levels of MAO-B in the brain and dopamine metabolism increase with aging, and consequently the level of reactive oxygen species. As a result, MAO-B has recently been proposed as an AD target, as neurons are especially sensitive to oxidative stress.[1]

Benzopyrone, a privileged structure valuable for the design and development of new chemical entities (NCE's), is the backbone of diverse natural or synthetic derivatives being linked to a vast array of pharmacological activities, for instance anti-inflammatory, antioxidant, anti-tumor and enzymatic inhibition.[2,3] Within this framework, coumarin (α-benzopyrone) and chromone (γ-benzopyrone) based compounds were synthesized and screened toward human monoamine oxidase isoforms (*h*MAO-A and *h*MAO-B). Mechanistic and computational docking studies have been performed to gain insight in the enzyme-inhibitor interactions and in a rationale for the observed selectivity and potency. The results of the comparative study will be presented in this communication.

References:

[1] Fonseca, A.; Matos, M. J.; Reis, J.; Duarte, Y.; Gutiérrez, M.; Santana, L.; Uriarte, E.; Borges, F. Exploring coumarin potentialities : development of new enzymatic inhibitors based on the 6-methyl-3- carboxamidocoumarin scaffold. *RSC Advances,* **2016**, *6*, 49764-49768.

[2] Gaspar, A.; Matos, M. J.; Garrido, J.; Uriarte, E.; Borges, F. Chromone: a valid scaffold in medicinal chemistry. *Chemical* Reviews, **2014**, *114*, 4960-4992.

[3] Borges, F.; Roleira, F.; Milhazes, N.; Uriarte, E.; Santana, L. Simple coumarins: Privileged scaffolds in medicinal chemistry. *Frontiers in Medicinal Chemistry*, **2009**, *4*, 23-85.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980). A.Fonseca (SFRH/BD/80831/2011) and J Reis (SFRH/BD/96033/2013) grants were also supported by FCT and FEDER/COMPETE funds.

Poster communication 14 - Working Group 1

Lipophilic properties of multiple ligands targeting cholinesterases and amyloid beta determined by micellar electrokinetic chromatography and reversed-phase thin-layer chromatography

Justyna Godyń,[a] Michalina Hebda,[a] Anna Więckowska,[a] Krzysztof Więckowski,[b] Barbara Malawska,[a] Marek Bajda[a]

[a] *Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Kraków, Poland*

[b] *Department of Organic Chemistry, Jagiellonian University Medical College, Kraków, Poland*

justyna.godyn@doctoral.uj.edu.pl

Alzheimer's disease (AD) is an irreversible, neurodegenerative disorder, considered to be the most common form of dementia, affecting 4-8% of the elderly population worldwide. The pathogenesis of AD is complex and many processes are involved in neuropathological changes leading to the neuronal death. Due to the multifaceted pathophysiology of AD and identification of many potential targets, an approach called multi-target directed ligand (MTDL) design seems to be an adequate one for search of new drugs. Thus, we decided to design new original MTDLs potentially acting as cholinesterase inhibitors (leading to the improvement of cholinergic transmission) and β-amyloide peptide (Aβ) aggregation inhibitors. In drug discovery process we focused both on the improvement of activity towards selected targets and the pharmacokinetic profile of new compounds, determined by their physicochemical properties. The presented work is a part of our studies on physicochemical properties of new MTDLs, especially on lipophilicity, considered to be the key drug-like property of the active compounds, which also seems to be essential for their activity in central nervous system. Among the fast and reliable methods for determination of lipophilicity of compounds micellar electrokinetic chromatography (MEKC) is considered to be an appropriate one for bioactive molecules, as it closely mimics the physiological conditions[1]. Thus, we used MEKC to estimate log P values for library of 49 derivatives of phthalimide, tetrahydroisochinoline and indole, designed and synthesized in our group as potential multiple anti-Alzheimer's agents with cholinesterase inhibitory activity and the Aβ anti-aggregation properties[2,3]. In addition, reversed-phase thin-layer chromatography (RP-TLC) method was applied for determination of R_{M0} values that are another lipophilicity descriptors. The results of both experimental methods were compared with each other and with computational methods (Marvin, ChemOffice Software). The lipophilicity-activity relationship was finally established, showing significant influence of lipophilicity on cholinesterase inhibition in some subgroups of phthalimide derivatives. The lipophilicity of all tested compounds was in a range defined by Lipinski rule-of-five (log P < 5), thus providing a good prognosis for intestinal permeability. Most of analyzed compounds did not exceeded log P = 3 value which is a criterion for a good blood-brain-barrier penetration[4].

References:

[1]Jia, Z., Physicochemical Profiling by Capillary Electrophoresis. *Current Pharmaceutical Analysis,* **2005**,1,41-56.

[2]Hebda M.; Bajda M.; et al., Synthesis, Molecular Modelling and Biological Evaluation of Novel Heterodimeric, Multiple Ligands Targeting Cholinesterases and Amyloid Beta. *Molecules,* **2016**,21,410.

[3]Więckowska A.; Więckowski K.; et al., Synthesis of new N-benzylpiperidine derivatives as cholinesterase inhibitors with β-amyloid anti-aggregation properties and beneficial effects on memory in vivo. *Bioorganic & Medicinal Chemistry,* **2015**,23,2445-2457.

[4]Pajouhesh, H.;Lenz, G.; Medicinal chemical properties of successful central nervous system drugs. *NeuroRX,* **2005**,2,541-553.

Poster communication 15 - Working Group 1

Tyrosinase inhibitors: synthesis, biological assays and docking studies of a new class of compounds

Laura Ielo,[a] Stefania Ferro,[a] Laura De Luca,[a] Maria Paola Germanò,[a] Maria Rosa Buemi,[a] Giovanna Certo,[a,b] M. Kanteev,[c] A. Fishman,[c] Antonio Rapisarda,[a] and Rosaria Gitto[a]

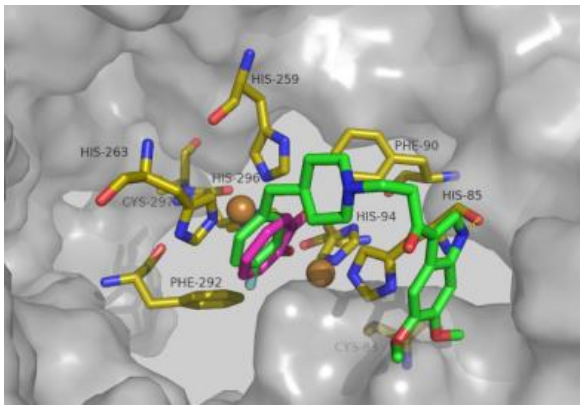
[a]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali (CHIBIOFARAM) Polo Universitario SS. Annunziata, Università di Messina, Viale Annunziata I-98168 Messina, Italy*

[b]*Fondazione Prof. Antonio Imbesi, Piazza Pugliatti 1, 98100 Messina, Italy*

[c]*Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa, 3200003, Israel*

lauielo@unime.it

Melanogenesis is a biosynthetic pathway for the formation of the pigment melanin in human skin. Tyrosinase (TYR) is a copper containing enzyme widely distributed in nature and it is the key enzyme of melanogenesis. It catalyses both the o-hydroxylation of monophenols and the subsequent oxidation of the resulting o-diphenols into o-quinones, which evolve spontaneously to produce melanin (1). Abnormal production of melanin causes various hyperpigmentary disorders and undesired browning of fruits and vegetables. A large number of TYR inhibitors have been reported in literature, but only a few are used because of their limitations with regards to cytotoxicity, selectivity and stability. This encourages researchers to seek more safety TYR inhibitors (2). Recently we identified a promising mushroom TYR inhibitor, 1-(5,6- dimethoxy-1H- indol-3-yl)-2- (4-(4-fluorobenzyl) piperidin-1yl)propan-1-one (1), showing an IC50 value comparable to kojic acid. Docking studies highlighted the ability of 1 to bind the active site of TYR (see Figure) (3). Starting from these results we designed a new series of TYR inhibitors, most of them displayed remarkable inhibitory activities. The obtained results together with the prediction of ADME properties suggested that some compounds are promising candidates for the treatment of tyrosinase-related disorders.



References:

[1] Liu, P.; Shu, C.; Liu, L.; Huang, Q.; Peng, Y. Design and synthesis of thiourea derivatives with sulfur-containing heterocyclic scaffolds as potential tyrosinase inhibitors. Bioorg Med Chem, 2016 24, 1866-71

[2] Zheng, Z.; Zhang, Y.; Zhang, S.; Chen, J. One-pot green synthesis of 1,3,5-triarylpentane-1,5-dione and triarylmethane derivatives as a new class of tyrosinase inhibitors. Bioorg Med Chem, 2016 26 795–798.

[3] Ferro S., De Luca L., Germanò MP., Buemi MR., Ielo L., Certo G., Kanteev M., Fishman A., Rapisarda A. and Gitto R. Chemical exploration of 4-(4-fluorobenzyl)piperidine fragment for the development of new tyrosinase inhibitors, Eur J Med Chem, accepted.

This work was supported by University of Messina Research & Mobility 2015 Project (Project Code: RES_AND_MOB_2015_DE_LUCA).

Poster communication 16 - Working Group 1

Salicylanilide carbamates active against Mycobacterium tuberculosis and other bacteria including drug-resistant strains

Martin Krátký,[a] Jarmila Vinšová,[a] Jiřina Stolaříková[b]

[a] *Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Kralove, the Czech Republic*

[b] *Laboratory for Mycobacterial Diagnostics and Tuberculosis, Regional Institute of Public Health, Partyzánské náměstí 7, 700 02 Ostrava, the Czech Republic*

martin.kratky@faf.cuni.cz; vinsova@faf.cuni.cz

Rapid emergence and spread of drug-resistant strains of *Mycobacterium tuberculosis* (*Mtb.*), non-tuberculous mycobacteria (NTM) as well as Gram-positive and Gram-negative bacteria[1] requires innovative therapeutic interventions. Unequivocally, the development of highly effective antimicrobial drugs with novel mechanism of action have become an insistent task.

We have synthesized a wide range of substituted salicylanilides (2-hydroxy-*N*-phenylbenzamides) exhibiting significant *in vitro* antibacterial activity, especially against *Mtb.*, NTM and Gram-positive bacteria including drug-resistant strains with a complex mechanism of action. However, their use in clinical practice is prevented by their comparatively higher toxicity and inconvenient physico-chemical properties, such as low solubility. Modifications of these parent molecules include protection of phenolic hydroxyl group that may help to overcome these undesired properties.[1]

Thus we synthesized series of salicylanilide carbamates[1,2,3,4] and thiocarbamates[4] (Fig. 1), where carbamic nitrogen is substituted by alkyl, cycloalkyl, aryl, arylalkyl or heteroaryl substituents. Parent salicylanilides were obtained by the reaction of salicylic acids and anilines with PCl_3 under MW irradiation.[1] *N*,*N*-Disubstituted (thio)carbamates were prepared *via* reaction of *in situ* generated salicylanilide triethylammonium salts with (thio)carbamoyl chlorides.[4] *N*-Monosubstituted carbamates were synthesized from salicylanilides by reaction with corresponding isocyanates in the presence of triethylamine.[1,2,3]

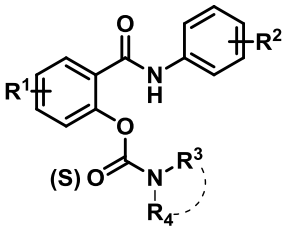


Figure 1: General structure of substituted salicylanilide (thio)carbamates.

These compounds exhibited very good *in vitro* activity against *Mtb.*, various strains of NTM, *Staphylococcus aureus* including methicillin-resistant strain or filamentous fungi with MIC values starting from ≤0.49 μM. Additionally, the most active derivatives were tested successfully against five clinically isolated MDR-TB strains with different resistance patterns and one XDR-TB strain without any decrease in the activity.

Salicylanilide *N*-monosubstituted carbamates exhibited a significant *in vitro* activity against various bacterial strains concomitantly with alleviated cytotoxicity when compared to salicylanilides.[1,2,3] The thiocarbamoylation did not improved antimycobacterial activity of parent molecules, but led to the considerably decreased cytotoxicity and thus favourable selectivity indexes. *N*,*N*-Disubstitution decreased antituberculosis effect when compared to *N*-monosubstitution which implies that the presence of hydrogen on the carbamoyl nitrogen is necessary for the excellent antimycobacterial activity.[4]

References:

[1] Krátký, M.; **Vinšová, J**. Salicylanilide *N*-monosubstituted carbamates: Synthesis and *in vitro* antimicrobial activity. *Bioorg. Med. Chem*., **2016**, *24*, 1322-1330.

[2] Férriz, J.M.; Vávrová, K.; Kunc, F.; Imramovský, A.; Stolaříková, J.; Vavříková, E.; **Vinšová,** J. Salicylanilide carbamates: Antitubercular agents active against multidrug-resistant Mycobacterium tuberculosis strains. *Bioorg. Med. Chem.*, **2010**, *18*, 1054-1061.

[3] Baranyai, Zs.; Krátký, M.; **Vinšová, J**.; Szabó, N.; Senoner, Z.; Horváti, K.; Stolaříková, J.; Dávid, S.; Bősze, S. Combating highly resistant emerging pathogen *Mycobacterium abscessus* and *Mycobacterium tuberculosis* with novel salicylanilide esters and carbamates. *Eur. J. Med. Chem.,* **2015***, 101*, 692-704.

[4] Krátký, M.; Volková, M.; Novotná, A.; Trejtnar, F.; Stolaříková, J.; **Vinšová, J**. Synthesis and Biological Activity of New Salicylanilide *N*,*N*-Disubstituted Carbamates and Thiocarbamates. *Bioorg. Med. Chem*., **2014**, *22*, 4073-4082.

Poster communication 17 - Working Group 1

Design, synthesis and biological evaluation of novel DNA gyrase B inhibitor-siderophore conjugates

Andraž Lamut,[a] Michaela Barančoková,[a] Janez Ilaš,[a] Päivi Tammela,[b] Danijel Kikelj[a] and Tihomir Tomašič[a]

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

[b] *Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland*

andraz.lamut@ffa.uni-lj.si

Infectious diseases remain one of the global health concerns due to increasing microbial resistance. Therefore, development of new antibacterial drugs with new mechanisms of action seems to be a promising concept of fighting against resistance. Bacterial topoisomerases represent one of the best established and validated targets in antibacterial drug discovery. DNA gyrase is type II topoisomerase and is composed of two GyrA (catalytic sites) and two GyrB (ATP-binding sites) subunits. Our research is focused on DNA gyrase B inhibitors and we have prepared a series of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole scaffold-based ATP-competitive inhibitors, which display enzyme inhibition in the low nanomolar range[1]. However, penetration of bacterial cell wall and efflux still seems to be a major problem according to *in vitro* evaluation of antibacterial activity of these inhibitors. To solve the problem of penetration, a Trojan horse concept using siderophores was applied. Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms and are among the strongest known soluble Fe^{3+} binding agents. Bacteria exploit siderophores to transport iron into the cytoplasm and use it as an essential nutrient. This mechanism could be used to design novel DNA gyrase B inhibitor-siderophore conjugates to improve antibacterial activity of inhibitors.

Recently, we have designed and prepared new conjugates of DNA gyrase B inhibitors with hydroxypiranone- and pyocheline-based siderophore mimics. Compounds were screened for their inhibitory activity in the *E. coli* DNA gyrase supercoiling assay. The most potent inhibitor with hydroxypiranone moiety possessed activity in the nanomolar range (IC_{50} = 140 nM, Figure 1). On the other hand, pyocheline-based siderophore conjugates showed weaker enzyme inhibition. We assume that pyochelin-like moiety is too bulky to fit the DNA gyrase B binding site. Evaluation of antibacterial activity of conjugates in a low-iron conditions test system is in progress and will reveal the true potential of this Trojan horse concept, which will guide the future optimization.

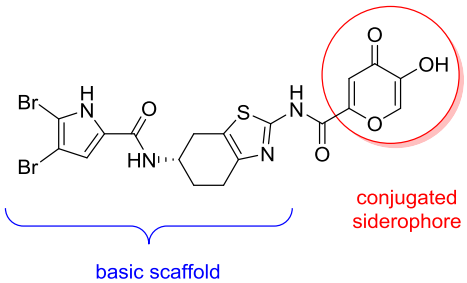


Figure 1: A representative structure of siderophore conjugated DNA gyrase B inhibitor.

References:

[1]Tomašič, T.; Katsamakas, S.; Hodnik, Ž.; Ilaš, J.; Brvar, M.; Šolmajer, T.; Montalvao, S.; Tammela, P.; Banjanac, M.; Ergović, G.; Anderluh, M.; Mašič, L.P.; Kikelj, D. Discovery of 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazoles as novel DNA gyrase inhibitors targeting the ATP-binding site. *J. Med. Chem*. **2015**, *58*, 5501-5521.

Poster communication 18 - Working Group 1

Optimizing Nature: development of novel antioxidants based on ferulic acid

Tiago Silva,[a] Daniel Martins,[a] Lisa Sequeira,[a] Daniel Chavarria,[a] Jorge Garrido,[b] Fernanda Borges[a]

[a] *CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal*
[b] *Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto, 4200-072 Porto, Portugal*

Danieljosemmartins@gmail.com

Oxidative stress is a very complex process that impacts biological systems in different aspects. The process is reliant on on the type of oxidant agent, on the site and intensity of its production, on the composition and concentration of endogenous antioxidants, and on the activity of repair systems. Oxidative stress can alter redox signaling in cells disrupting the normal homeostasis, which in some cases can lead to major cellular damage, thus being connected with a number of diseases, namely those associated with aging. In a pathological event, the pool of endogenous antioxidant defences may not be enough to deal with the increased level of oxidant species production. In that sense, the administration of exogenous antioxidants can be beneficial to decrease cell injury, compensating the insufficiency of endogenous defence systems and improving the overall antioxidant response. Exogenous antioxidants may block the complex networks of oxidative damage pathways at different levels, yielding a therapeutic effect. Consequently, antioxidants that are exogenously acquired from diet may have important functions in redox cell homeostasis and can be important for cellular function and disease prevention. Antioxidants may exert their effects by different mechanisms, such as neutralizing circulating reactive species (scavenging activity), sequestering transition metal ions (chelation activity) and inhibiting enzymes involved in the production of reactive species.

Phenolic compounds represent a structurally diverse group of plant secondary metabolites and have been attracted the interest from the researchers due to their assorted biological outlines (*e.g.* antioxidant, antiallergic, anti-inflammatory, antimicrobial, antiviral, anticarcinogenic and neuroprotective activities). Phenolic acids are one of the major classes of phenolic compounds and are widely distributed in fruits, vegetables, coffee, wine, beer and olive oil. Phenolic acids are classified as hydroxycinnamic acids (HCAs) or benzoic acids according to the presence or absence of a double bond with *trans* configuration between the aromatic ring and the carboxylic acid, respectively. Although phenolic acids have been showing considerable antioxidant activity *in vitro* they have bioavailability drawbacks. Over the last years, the lipophilicity of phenolic acids has been increased by structural refinement, for instance, the lipophilicity of HCAs have been increased by esterification or amidation of carboxylic acid function. In this communication, the synthesis of lipophilic HCA derivatives based on the ferulic acid scaffold as well as the evaluation of their physicochemical and antioxidant properties using *in vitro* cell-free and cell-based assays will be reported.

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 (SFRH/BD/108119/2015) grant was also supported by FCT and FEDER/COMPETE funds.

References:

Menezes, J.; Kamat, S.; Cavaleiro, J.; Gaspar, A.; Garrido, J.; Borges, F. *Eur. J. Med. Chem,* **2011,** *46*, 773-777.

Teixeira, J.; Gaspar, A.; Garrido, E.; Garrido, J.; Borges, F.; *Biomed Res. Int.*, **2013**, *2013,* 1-11.

Poster communication 19 - Working Group 1

Symbiotic approach applied to the synthesis of new agents for the treatment of Alzheimer's disease

Giulia Nesi,[a] Shengnan Wang,[b] Simona Sestito,[a] Chiara Barbieri,[a] Ziwei Chen,[b] Xiaohong Yang,[b] Rongbiao Pi,[b] Simona Rapposelli[a]

[a] *Dept of Pharmacy, University of Pisa, via Bonanno, 6, 56126, Pisa, Italy*

[b] *School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China*

giulia.nesi@farm.unipi.it

Alzheimer disease (AD) is a progressive age-dependent neurodegenerative pathology. The number of people with AD is steadily increasing and has a severe impact on individuals, families, and society. Although the etiology of AD is not yet completely explored, it is now commonly established that it is a multifactorial neurodegenerative pathology. Nowadays, many agents have been synthesized for the treatment of memory and cognition impairments able to interact with different targets. Unfortunately, AD therapy still lack in effectiveness therefore, the search for new potential drugs is heavily pursued. Consistently, the search for new multitarget agents has led us to design and synthesize a series of tacrine–hydroxycinnamic acid (caffeic or ferulic acid) hybrids that showed acetylcholinesterase (AChE) inhibitory activity and antioxidants properties[1]. Recently, with the aim of increasing the affinity for the butyrylcholinesterase (BuChE) we reported the synthesis and in vitro activities of novel rivastigmine-hydroxycinnamic acid hybrids as inhibitors of AChE and BuChE, ROS scavengers and inhibitors of Aβ aggregation[2]. In order to expand the SAR study for this new class of compounds, herein we describe the design and synthesis of analogs **1-6** obtained by the symbiotic combination of rivastigmine skeleton with natural antioxidant agents such as gallic acid and 2-chromonecarboxylic acid. The new ligands were evaluated in different systems for neuroprotective, antioxidant, ChE inhibitory and inhibitory of Aβ aggregation properties.

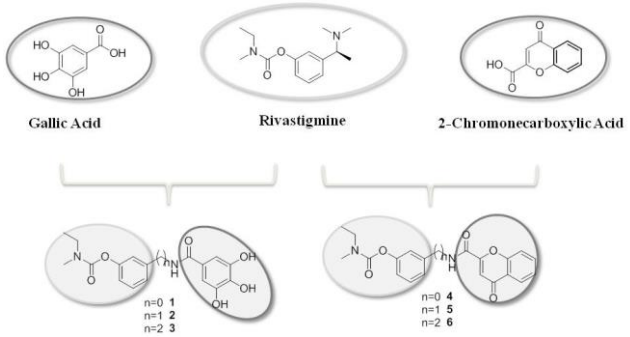


Figure 1: Symbiotic approach for the synthesis of new multitarget anti-AD agents.

References:

[1] Digiacomo, M.; Chen, Z.; Wang, S.; Lapucci, A.; Macchia, M.; Yang, X.; Chu, J.; Han, Y.; Pi, R.; Rapposelli, S., Synthesis and pharmacological evaluation of multifunctional tacrine derivatives against several disease pathways of AD. *Bioorganic & medicinal chemistry letters,* **2015**, *25*, (4), 807-810.

[2] Chen, Z.; Digiacomo, M.; Tu, Y.; Gu, Q.; Wang, S.; Yang, X.; Chu, J.; Chen, Q.; Han, Y.; Nesi, G.; Sestito, S.; Marco, M.; Rapposelli, S.; Pi, R. Discovery of novel rivastigmine-hydroxycinnamic acid hybrids as multi-targeted agents for Alzheimer's disease, *European Journal of Medicinal Chemistry*, **2016**, DOI: 10.1016/j.ejmech.2016.09.052

Poster communication 20 - Working Group 1

Cyclopentenediones as a relatively new group of bioactive substances

David Novak, Eva Kocianova and Jan Vacek

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 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, 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.[1] The main goal of this contribution is to highlight a novel library of CPDs based on 2-benzylidene substitution[2] and evaluate the application potential of selected CPDs, *e.g.* dimeric CPD nostotrebin 6 with antibacterial properties.[3]

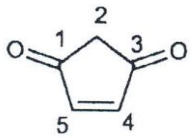


Figure 1: General structure of CPD.

References:

[1] Sevcikova, Z.; Pour, M.; Novak, D.; Ulrichova, J.; Vacek, J. Chemical Properties and Biological Activities of Cyclopentenediones: A Review. *Mini-Rev. Med. Chem.*, **2014**, *14*, 322-331.

[2] Papouskova, B.; Bernard, M.; Ottenschlager, J.; Karban, J.; Velisek, P.; Hrbac, J.; Sykora, J.; Storch, J.; Vacek J. A comprehensive LC/MS analysis of novel cyclopentenedione library. *J. Pharm. Biomed. Anal.*, **2016**, 128, 342–351.

[3] Cheel, J.; Bogdanova, K.; Ignatova, S.; Garrard, I.; Hewitson, P.; Kolar, M.; Kopecky, J.; Hrouzek, P.; Vacek, J. Dimeric cyanobacterial cyclopent-4-ene-1,3-dione as selective inhibitor of Gram-positive bacteria growth: Bio-production approach and preparative isolation by HPCCC. *Algal Res.*, **2016**, 18, 244–249.

Poster communication 21 - Working Group 1

Benzoic based amide nitrones: a new class of cholinesterase inhibitors endowed with neuroprotective activity

Catarina Oliveira,[a] Fernando Cagide,[a] Jorge Garrido,[a,b] Fernando Remião,[c] Eugenio Uriarte,[c] 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]*Department of Chemical Engineering, Superior Institute of Engineering of Porto (ISEP), IPP, Rua Dr. António Bernardino de Almeida, 431, 4200-072, Porto, Portugal;*
[c] *UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*
[d]*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.*

catarina.oliveira.fc@gmail.com

Aging process is intrinsically associated with a progressive decline in several biologically relevant functional pathways, which in turn can significantly increase the risk of developing neurodegenerative and other age-related diseases. Alzheimer's disease (AD) is the most prevalent type of dementia. AD is currently associated to cell-altered oxidative stress status, a processs that is related with a failure in the antioxidant protective system and/or an increment in reactive species production/accumulation. Overall it can cause the destabilization of cellular membranes, damage of blood-brain-barrier (BBB), disintegration of DNA and ultimately, neuronal death.

In this context, neuroprotective agents with an extended therapeutic window are urgently needed. As neuroprotection efficacy depends to a great extent on the chemical connectivity and the nature of substituents, the aim of this project has been focused on the design and synthesis of innovative lipophilic hybrid arylnitrones using benzoic acid as a scaffold. Nitrones are known to be spin traps that have the ability to stabilise or trap free radicals. In this context they are also able to reduce the damage associated with unbalanced radical production.

In the present project we have followed a hybridization strategy that encloses the introduction of fragments able to display neuroprotective properties, such as the nitrone moiety, on benzoic scaffold ,and lipophilic linkers that can confer the required lipophilicity to cross BBB. After synthesis, purification and structural identification, the novel compounds have been screened toward cholinesterase enzymes (AChE and BChE) as they are a key AD target. Their cytotoxic and neuroprotective profile was evaluated SH-SY5Y neuroblastoma cell liness. 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/00081/2015, POCI-01-0145-FEDER-006980 and NORTE-01-0145-FEDER-000028). Catarina Oliveira (SFRH/BD/88773/2012) and Fernando Cagide (SFRH/BPD/74491/2010) were also supported by FCT and FEDER/COMPETE funds.

Poster communication 22 - Working Group 1

Synthesis of a disaccharide rigid lanthanide binding tag to aid NMR studies of a coat protein of human norovirus

Jose M. Orduña,[a] Alvaro Mallagaray,[b] Gema Domínguez,[a] Thomas Peters[b] and Javier Pérez-Castells[a]

[a]*Department of Chemistry and Biochemistry, Faculty of Pharmacy, University San Pablo CEU, Urb. Montepríncipe, Ctra. Boadilla del Monte, Km. 5.300, 28668 Madrid, España.*
[b]*Institute of Chemistry, Center for Structural and Cell Biology in Medicine (CSCM) University of Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany.*

josemaria.ordunamendez@ceu.es

Paramagnetically tagged carbohydrates give access to information on the conformation of the sugar and on the binding site, not easily available from other experimental techniques [1]. Attachment of human noroviruses to histo blood group antigens (HBGA) is thought to be essential for infection of host cells. Molecular details of the attachment process can be studied *in vitro* using a variety of NMR experiments and the use of lanthanide tags non-covalently binding to viral coat proteins such as P-dimers offers a strategy that complements classical assignment approaches based on ^{2}H,^{13}C,^{15}N labeling and corresponding triple resonance 3D NMR experiments. This approach may be instrumental in obtaining a backbone assignment of this demanding 70 kDa protein target in the future. We have shown [2] that an □-L-fucose derivative carrying a chelating unit for lanthanide ions serves as a non-covalent paramagnetic probe to assist assignment of norovirus P-dimers using PRE and PCS data to aid backbone assignment [3].

Herein we show the synthesis of a new tag including a disaccharide present in the HBGAs. This new tag includes a different chelating unit with higher coordination number[4].

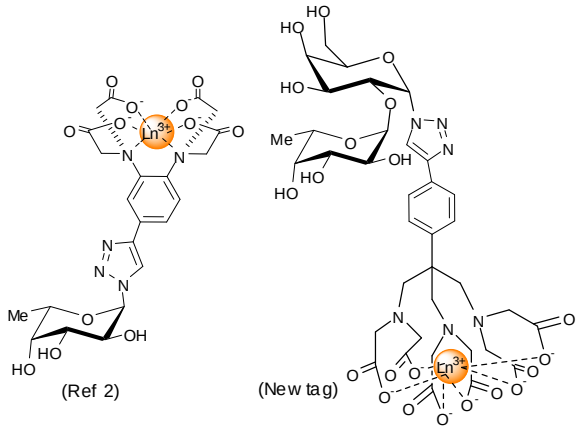


Figure 1: Tag previously synthesized by our group [2], and the new tag proposed.

The synthesis has been accomplished in a modular way from β-D-Galactose pentaacetate, 2,3,4-trisbenzyl-1-ethylthio-α-L-fucose and the chelating unit using as the key step a click reaction.

References:

[1] Canales, A.; Mallagaray, A.; Alvaro Berbis, M.; Navarro-Vazquez, A.; Dominguez, G.; Javier Canada, F.; Andre, S.; Gabius, H.-J.; Perez-Castells, J.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **2014,** *136*, 8011-8017; Erdelyi, M.; d'Auvergne, E.; Navarro-Vazquez, A.; Leonov, A.; Griesinger, C. *Chem. Eur. J.* **2011,** *17*, 9368-9376; Mallagaray, A.; Canales, A.; Dominguez, G.; Jimenez-Barbero, J.; Perez-Castells, J. *Chem. Commun.* **2011,** *47*, 7179-7181; Yamamoto, S.; Yamaguchi, T.; Erdelyi, M.; Griesinger, C.; Kato, K. *Chem. Eur. J.* **2011,** *17*, 9280-9282; Zhang, Y.; Yamamoto, S.; Yamaguchi, T.; Kato, K. *Molecules* **2012,** *17*, 6658-6671.

[2] Mallagaray, A.; Dominguez, G.; Peters, T.; Perez-Castells, J. *Chem. Comm.* **2016,** *52*, 601-604.

[3] S. P. Skinner, M. Moshev, M. A. S. Hass and M. Ubbink, *Journal of Biomolecular NMR*, 2013, **55**, 379-389; G. Pintacuda, M. A. Keniry, T. Huber, A. Y. Park, N. E. Dixon and G. Otting, *Journal of the American Chemical Society*, 2004, **126**, 2963-2970.

[4] Kato, K.; Yamaguchi, T. *Glycoconjugate J.* **2015,** *32*, 505-513.

Acknowledgements

Funding of this project by Spanish MINECO (No. CTQ2015-64624-R; FEDER/MINECO) and FUSP-CEU (PC17/15) is acknowledged. J. M. thanks the Comunidad Autonoma de Madrid and the Youth Guarantee System for a research assistant grant. A.M. is recipient of a Marie Curie fellowship within the 7th Framework Program of the EU (Grant Agreement No. 329485, NoroCarb). T.P. acknowledges continuing support by the University of Lubeck.

Poster communication 23 - Working Group 1

Multi Target Drug Discovery ¨MTDD¨ a new approach for the treatment of cancer

M. Pastor-Fernández, José María Zapico, Beatriz de Pascual-Teresa, Ana Ramos

[a] *Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU San Pablo, 28668 Boadilla del Monte, Madrid, Spain.*

miryam.pastorfernandez@ceu.es

Chemotherapeutics history informs us that Paul Ehrlich, with his "*magic bullet*" concept, inspired the era of designing "*target selective*" drug molecules.[1] Nowadays, Multi-target Drug Discovery (MTDD) has emerged as an area of increasing interest to the drug discovery community. Drugs that modulate several targets have the potential to improve the efficacy and safety of single target agents. The challenge of MTDD lies in obtaining molecules with the appropriate and balanced affinities for selected multiple receptors. In this work, we describe the design and synthesis of HDAC1/CK2 and MMP2/CK2 dual inhibitors, with potential antitumor activity.[2]

Recently[3], our research group has published a first example of HDAC1/CK2 inhibitors obtained using a clik chemistry approach. The rationale of their design was based on the knowledge that CK2 is involved in the activation of histone deacetylase type 1 (HDAC1) in hypoxia-associated cancer processes, thus contributing to tumor angiogenesis.[4] A study on the binding mode of the described ligands allowed us to propose a new series of compounds which have been synthesize and tested for their affinity to both enzymes.

On the other hand, our group was able to demonstrate that Matrix Metaloproteinase 2 (MMP2) is a substrate of CK2 and that selective inhibition of protein kinase CK2 in HT1080 cells results in up-regulation of MMP2.[5] MMP2 is involved in tumour growth, angiogenesis and metastasis. Following the same click chemistry approach, and based on our experience in the design of inhibitors for both enzymes, we have synthesized a series of new compounds, designed to act as dual targeting MMP2/CK2 inhibitors.

References:

1. Strebhardt, K.; Ullrich, A., Paul Ehrlich's magic bullet concept: 100 years of progress. *Nat Rev Cancer* **2008,** *8* (6), 473-80.

2. Donald, S., The Multi-Target Drug Design Era is Here, Consider it. *Drug Design* **2012,** *1* (1).

3. Purwin, M.; Hernandez-Toribio, J.; Coderch, C.; Panchuk, R.; Skorokhyd, N.; Filipiak, K.; de Pascual-Teresa, B.; Ramos, A., Design and synthesis of novel dual-target agents for HDAC1 and CK2 inhibition. *RSC Advances* **2016,** *6* (71), 66595-66608.

4. Ropero, S.; Esteller, M., The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* **2007,** *1* (1), 19-25.

5. Filipiak, K.; Kubiński, K.; Hellman, U.; Ramos, A.; de Pascual-Teresa, B., Human protein kinase CK2 phosphorylates matrix metalloproteinase 2 and inhibits its activity. *Chembiochem* **2014,** *15* (13), 1873-6.

Acknowledgments. This work is supported by (CTQ2014-52604-R) and (CEU MPC 15/11)

Poster communication 24 - Working Group 1

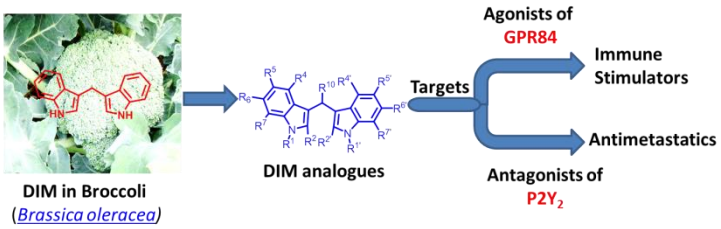
Diindolylmethanes (DIMs): from nutrients to multitarget anti-cancer drugs

Thanigaimalai Pillaiyar, Meryem Köse, Isaac Attah, and Christa E. Müller

PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

thanigai@uni-bonn.de; christa.muller@uni-bonn.de

Cruciferous vegetables (*Cruciferae*) are a rich source of vitamin C, soluble fiber and multiple nutrients and phytochemicals. Cruciferous vegetables contain glucosinolates which have potential anti-cancer activity. In particular those of the *Brassica* genus, such as broccoli, brussel sprouts, cabbage, and cauliflower are reportedly associated with a reduced risk of breast, prostate and colorectal cancer. 3,3'-Diindolylmethane (DIM) is a natural indole derived from its precursor indole-3-carbinol which is found in large amounts in cruciferous vegetables such as broccoli. The effects of DIM include inhibition of estrogen-induced growth of breast cancer cell lines (*via* estrogen receptor) and human breast tumors. DIM has also been reported to activate the arylhydrocarbon receptor (AhR), a ligand-activated transcription factor associated with gastric carcinogenesis. Recently, DIM was identified and characterized as the first synthetic agonist for the orphan G protein-coupled receptor (GPCR) GPR84.[1] GPR84 is a class A G_i protein-coupled δ-branch receptor activated by medium chain (hydroxy)fatty acids. It is highly expressed in peripheral immune cells and microglia in the CNS, and has therefore been proposed to play an important role in the immune system.



DIM derivatives and analogs as multitarget drugs

Structure-activity relationship (SAR) analysis of DIM derivatives as GPR84 agonists allowed their optimization and led to the development of agonists with improved potency. In an effort to develop potent ligands for multiple targets, DIMs were screened at various δ-branch GPCRs potentially relevant for cancer therapy. Interestingly we found that DIMs were able to block the purinergic GPCR $P2Y_2$ at low micromlar concentrations. Recent findings showed that $P2Y_2$ receptor blockade may be a promising strategy for anti-metastatic therapy.[2] We therefore propose multi-target anti-cancer drugs based on the DIM scaffold which activate GPR84 thereby stimulating the immune system to eliminate tumour cells, and which at the same time block $P2Y_2$ receptors resulting in an anti-metastatic effect.

References:

[1] Takeda, S.; Yamamoto, A.; Okada, T.; Matsumura, E.; Nose, E.; Kogure, K.; Kojima, S.; Haga, T. Identification of surrogate ligands for orphan G protein-coupled receptors. *Life Sci*. **2003**, *74*, 367–377.

[2] Schumacher, D.; Strilic, B.; Sivaraj, KK.; Wettschureck, N.; Offermanns, S. Platelet-derived nucleotides promote tumor-cell transendothelial migration and metastasis via P2Y2 receptor. *Cancer Cell* **2013**, *24*, 130-137.

Poster communication 25 - Working Group 1

Pteridine derivatives as potent PTR1 inhibitors for the treatment of Trypanosomiasis and Leishmaniasis

Antonio Quotadamo,[a] Anabela Cordeiro-da-Silva,[b] Sheraz Gul,[c] Rebecca C. Wade,[d] Ina Poehner,[d] M. P. Costi,[a] Alberto Venturelli[e]

[a]*University of Modena and Reggio Emilia, Via Campi 103, 41125, Modena, Italy.*

[b]*IBMC , Universidade do Porto, Oporto, Portogual.*

[c]*Fraunhofer-IME SP, Schnackenburgallee 114, D-22525 Hamburg, Germany.*

[d]*Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany.*

[e]*Tydock Pharma srl, Strada Gherbella 294/b, 41121 Modena, Italy,*

antonio.quotadamo@unimore.it

Drugs currently in use against Leishmania and Trypanosoma infections have limitations in terms of efficacy, toxicity and resistance. It is therefore mandatory to identify molecular targets to be specifically inhibited. Folate-dependent enzymes targeting drugs should be useful against diseases from trypanosomatids infections. However, the classical inhibitors of dihydrofolate reductase (DHFR) are ineffective. The unusual primary resistance of trypanosomatids against anti-folates can be explained by overlapping activities of pteridine reductase (PTR1). PTR1, by its ability to provide reduced pterins and folates, has the potential to act as a by-pass DHFR inhibition under physiological conditions. A combined inhibition of PTR1 and DHFR-TS activity leads to efficacy eradication of the parasite and is therefore considered to be a promising approach to treat trypanosomatidic diseases.

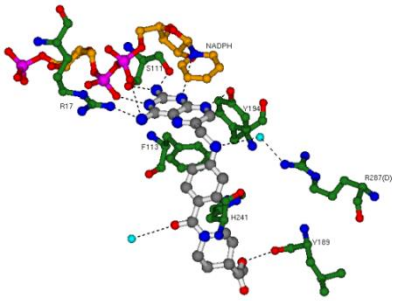


Figure 1: Pteridine compound binding to PTR1 enzyme.

In this contest, we focus on the synthesis of novel pteridine compounds, structure correlated to Methotrexate (MTX), able to inhibit only PTR1 or with dual inhibition approach. To achieve this aim, the rational design is performed on the biological profile of the tested compounds against the target proteins and optimized by computation and crystallographic studies. The synthesized compounds have been evaluated for their capability to inhibit PTR1 enzyme, with an optimal activity and selectivity against the target enzymes (IC_{50} in the low nM range) and a safe in vitro ADME profile. These compounds have been tested on parasite cells. Some of them showed an interesting synergistic activity in combination with MTX (DHFR inhibitor). Five compounds have been tested towards *T. brucei* with an EC_{50} lower than 4 µM are under PK proprieties evaluation.

References:

[1] Cavazzuti, A., et al., *Discovery of potent pteridine reductase inhibitors to guide antiparasite drug development*. Proc Natl Acad Sci U S A, 2008. **105**(5): p. 1448-53.

This work was supported by PRIN 2009-2011 to MPC and NMTrypI (New medicine for Trypanosomatidic Infections). This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no.603240. www.nmtrypi.eu

Poster communication 26 - Working Group 1

Microwave assisted synthesis and antibacterial evaluation of 3-aminonpyrazine-2-carboxamide derivatives

Lucia Semelková,[a] Carlos Fernandes,[b] Klára Konečná,[c] Pavla Paterová,[d] Martin Doležal[a]

[a] *Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Ak. Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*

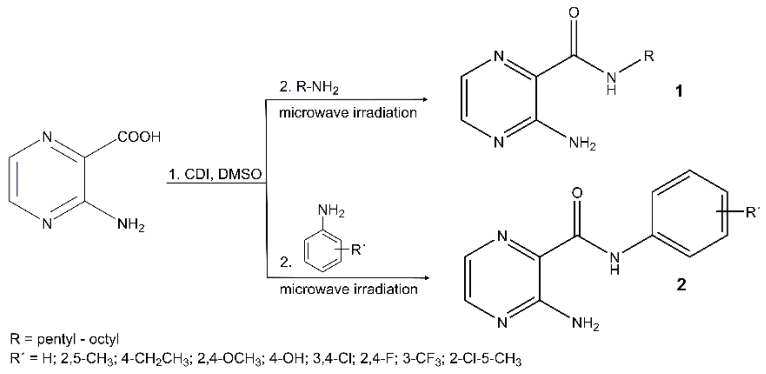
[b] *Faculty of Pharmacy in Porto, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

[c] *Department of Clinical Microbiology, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic*

semelkol@faf.cuni.cz

Pyrazinamide (PZA) is an important first-line antitubercular drug. PZA is prodrug, which is converted to its active form pyrazinoic acid (POA). POA accumulates inside the cell which leads to acidification of cytoplasm[1]. Other targets are connected to specific enzymes – RpsA (participated in trans-translation process)[2] and aspartate decarboxylase (involved in energy metabolism)[2]. 5-chloropyrazinamide derivatives and propyl esters of POA inhibit fatty acid synthase I (synthesis of mycolic acids).[1]

Series of *N*-alkyl-3-aminopyrazine-2-carboxamides (**1**) and substituted 3-amino-*N*-phenylpyrazine-2-carboxamides (**2**) were synthetized in this work.



Prepared compounds were tested *in vitro* for their antimycobacterial, antibacterial and antifungal activity. 3-Amino-*N*-octylpyrazine-2-carboxamide exerted moderate activity against *Mycobacterium tuberculosis* and *M. avium* (MIC = 50 µg/mL) and interesting activity against *M. kansasii* (MIC = 25 µg/mL).

The most active compound against *Candida albicans* was 3-amino-*N*-(4-ethylphenyl)pyrazine-2-carboxamide with MIC = 7.81 µmol/L and 3-amino-*N*-(3-(trifluoromethyl)phenyl)pyrazine-2-carboxamide was the most effective substance against methicillin resistant *Staphylococcus aureus* with MIC = 1,95 µmol/L.

The study was supported by the Grant Agency of Charles University, project B–CH 1594214 and SVV 260 291.

References:

[1] Singh, P., Mishra, A. K., Malonia, S. K. et al.: The paradox of pyrazinamide: an update on the molecular mechanisms of pyrazinamide resistance in Mycobacteria. J Commun Dis., **2006**, 38, 288-298.

[2] Shi, W., Chen, J., Feng, J. et al.: Aspartate decarboxylase (PanD) as a new target of pyrazinamide in Mycobacterium tuberculosis. Emerging Microbes & Infections, **2014**, 3, e58.

Poster communication 27 - Working Group 1

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 de Synthése et Catalyse (UMR CNRS 7509), 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 by us 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)

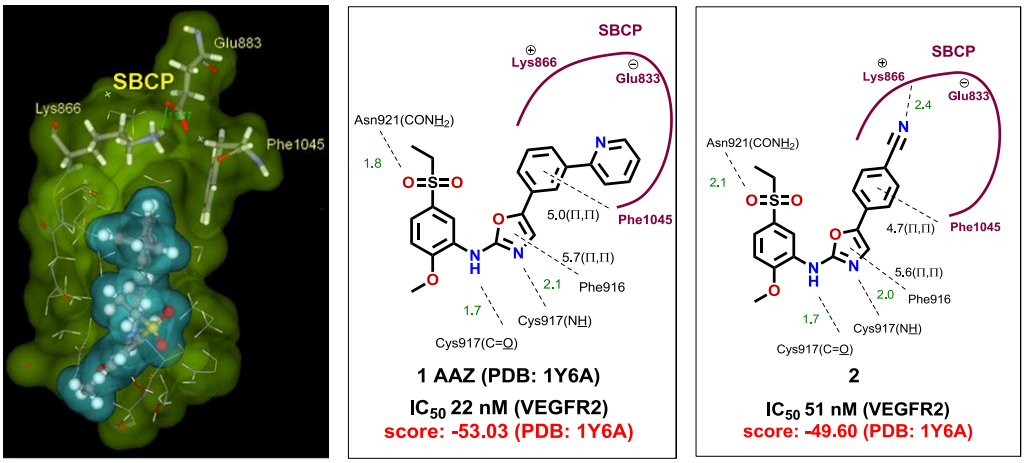


Figure 1 The left picture represents 3D visualization of AAZ (1) in VEGFR2 TK binding place with highlighted SBCP arrangement. The second and third picture represent binding interaction maps of 1 (AAZ) and one predicted for the previously prepared para substituted derivative (2) with SBCP domain.

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)

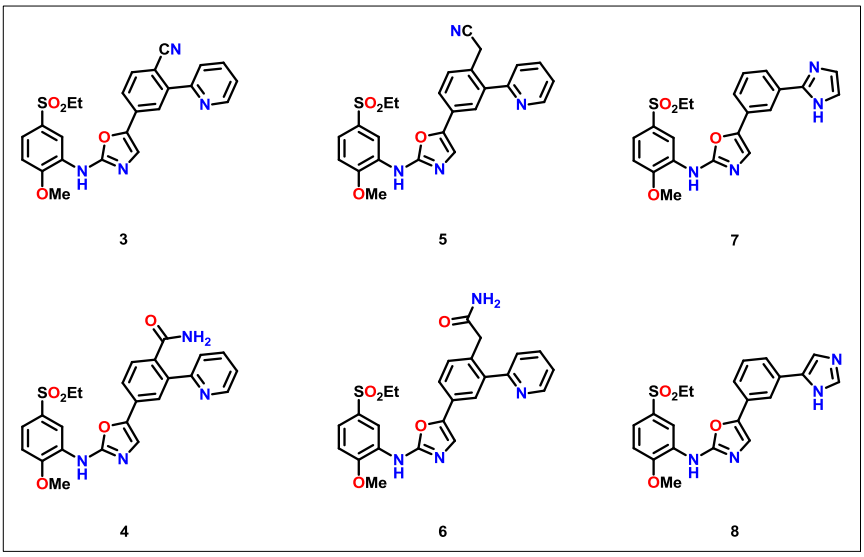


Figure 2 The designed AAZ-based TKIs (3, 4, 5, 6, 7, 8).

References:

[1] GlaxoSmithKline, Five Moore Drive, Research Triangle Park, North Carolina 27709, USA.

[2] Harris, P. A.; Cheung, M.; Hunter, R. N.; Brown, M. L.; Veal, J. M.; Nolte, R. T.; Wang, L.; Liu, W.; Crosby, R. M.; Johnson, J. H.; Epperly, A. H.; Kumar, R.; Luttrell, D. K.; Stafford, J. A. *J. Med. Chem.* **2005**, *48*, 1610 – 1619.

Poster communication 28 - Working Group 1

Target and sub-target identification and modulation using the bimolecular approach

Pavel Starkov,[a] Rohit Bhadoria[a]

[a] *Dept of Chemistry & Biotechnology, Tallinn University of Technology, 12618, Tallinn, Estonia*

pavel.starkov@ttu.ee

Small molecules are the pinnacle of modern biomedicine. Many high-throughput and high-content screening campaigns have to date delivered numerous collections of small molecules with so called interesting phenotypes. In many cases – perhaps, if not in the majority thereof – their precise role in guiding or disturbing biological processes, however, remains unclear.

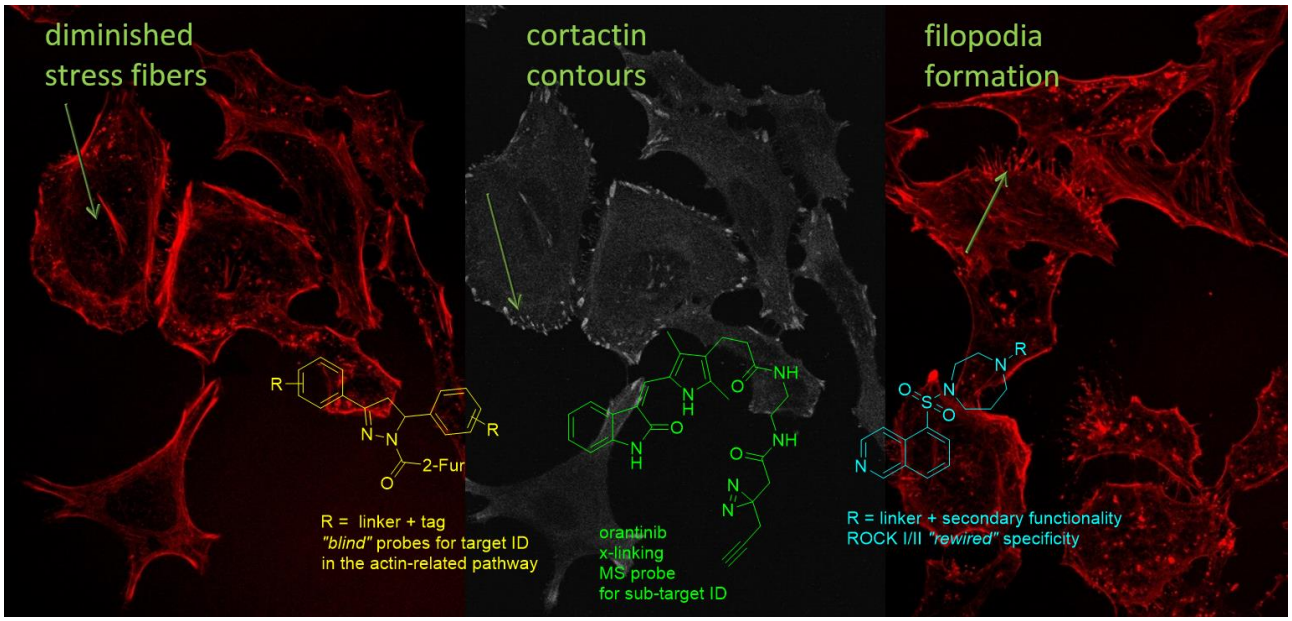


Figure 1: Target and sub-target identification and modulation using the bimolecular approach.

Our lab has set out a programme on how to identify and use additional modalities and functions-encoding units in order to investigate target validation/modulation using chemical approaches. We are developing both “smart”-tagging strategies to identify potential biological cross-target subsets of existing pool of small molecules and looking into how to ascertain “guided” specificity of the existing ones.

Poster communication 29 - Working Group 1

Novel N-Phenyl-4,5-dibromopyrrolamides and N-Phenyl-3,4-dichloro-5-methylpyrrolamides Targeting DNA Gyrase B and Topoisomerase IV

Davide Benedetto Tiz,[a] Danijel Kikelj[a] and Nace Zidar[a]

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

Davide.Benedetto.Tiz@ffa.uni-lj.si

DNA gyrase and topoisomerase IV are enzymes involved in the winding and unwinding of the supercoiled helixes of bacterial DNA. Both, DNA gyrase and topoisomerase IV belong to type IIA topoisomerases which catalyze the transient break of two strands of DNA. Another common characteristic of these two enzymes is the heterotetrameric structure: DNA gyrase is composed of two subunits called GyrA and two subunits called GyrB, while topoisomerase IV is made up of two ParC subunits (homologous to GyrA) and two ParE subunits (homologous to GyrB). GyrA/ParC subunit plays an important role in the cleavage and reassembly of DNA whereas the main role of GyrB/ParE subunit is the hydrolysis of ATP which affords the energy for the cleavage-ligation process. [1]

One of the first well-known examples of DNA gyrase B inhibitors is novobiocin, an aminocoumarin antibiotic belonging to the golden age of antibacterials.

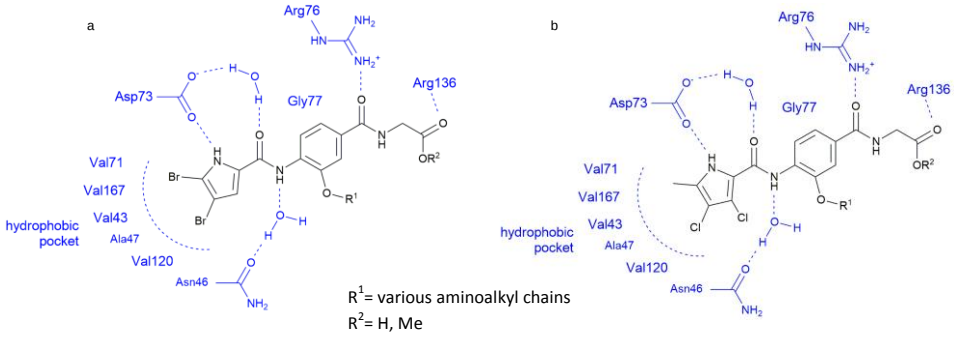


Figure 1: Two series of *N*-phenyl-pyrrolamides are shown including the key ligand-enzyme interactions for both *N*-phenyl-4,5-dibromopyrrolamides (a) and *N*-phenyl-3,4-dichloro-5-methylpyrrolamides (b). Numbering of AA is according to *E. coli*.

Starting from previous "*in house*" scaffolds, we have designed, synthesized and evaluated a new series of molecules against DNA gyrase and topoisomerase IV on both Gram- and Gram+ strains (**Figure 1**).

One of the synthesized compounds, 4-(5-((carboxymethyl)carbamoyl)-2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)phenoxy)piperidin-1-ium chloride, showed an excellent IC_{50} value (50 nM) but the antibacterial activities of all the compounds were relatively low. Thus, an optimization of the physicochemical properties (e.g., the isosteric replacement of the carboxylic acid with a more lipophilic heterocycle) is required in order to obtain a significant penetration and activity in the bacterial cells.

References:

[1]Zidar, N.; Macut, H.; Tomašič, T. et al. N-Phenyl-4,5-dibromopyrrolamides and *N*-Phenylindolamides as ATP Competitive DNA Gyrase B Inhibitors: Design, Synthesis, and Evaluation. *J. Med. Chem*, **2015**, *58*, 6179−6194.

Poster communication 30 - Working Group 2

First report of the *in vitro* nematicidal effects of camel milk

Hafidh Akkari,[a] Dhouha Alimi,[a,b] Soumaya Hajaji,[a,b] Mourad Rekik,[c] Amel Abidi,[a] Mohamed Gharbi[a]

[a] Laboratoire de Parasitologie, Université de la Manouba, École Nationale de Médecine Vétérinaire de Sidi thabet, 2020,Tunisie

[b] Faculté des sciences de Bizerte, Université de Carthage, 7021, Zarzouna, Tunisie

[c] International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box, 950764 Amman 11195 Jordan

hafidh_akkari@yahoo.fr

Antipathogenic properties of camel milk have been investigated to substitute for drugs hence overcome drug resistance. The main objective of this present study was to investigate the anthelmintic activity of camel milk in relationship to its chemical composition. *In vitro* anthelmintic effects of camel milk against *Haemonchus contortus* from sheep were ascertained by egg hatching and worm motility inhibitions in comparison to milks from cow, ewe and goat as well as a reference drug albendazole. Chemical composition revealed that camel milk has higher contents of protective protein (lactoferrin) and vitamin C than other species' milk. It showed ovicidal activity at all tested concentrations and completely inhibited egg hatching at a concentration close to 100 mg/mL (inhibitory concentration (IC_{50}) = 42.39 mg/mL). Camel milk revealed *in vitro* activity against adult parasites in terms of the paralysis and/or death of the worms at different hours post treatment. After 8 h of exposure, it induced 100% mortality at the highest tested concentration. There was 82.3% immobility of worms in albendazole 8 h post-exposition. No such effects were seen with the other species' milks. Bioactive compounds such as lactoferrin and vitamin C may be involved in such an effect.

To our knowledge, these results depict for the first time that camel milk possesses *in vitro* anthelmintic properties and further *in vitro* and *in vivo* trials against different parasite species and stages are required to make use of this milk for the control of gastrointestinal nematode parasites.

Poster communication 31 - Working Group 2

Studies on Inclusion complexes of cyclodextrins

Adina Arvinte,[a] Narcisa Marangoci,[a] Bogdan Minea,[a] Mariana Pinteala[a]

[a] *"Petru Poni" Institute of Macromolecular Chemistry, Centre of Advanced Research in Bionanoconjugates and Biopolymers, Grigore Ghica Voda Alley 41A, 700487 Iasi, Romania*

adina.arvinte@icmpp.ro

In the concept of smart drug delivery systems able to release the therapeutic load "on demand", biocompatible and biodegradable compounds represent a promising approach. Different carrier materials are constantly developed to overcome the undesirable properties of drug molecules [1]. Among them, cyclodextrins (CDs) have been found as potential candidates because of their ability to alter physical and chemical properties of guest molecules through the formation of inclusion complexes, meanwhile to increase the aqueous solubility of poorly soluble drugs and to increase their bioavailability and stability. As a result of their molecular structure and shape, CDs act as molecular containers by entrapping guest molecules in their internal cavity. No covalent bonds are formed or broken during drug-CD complex formation, and in aqueous solution, the complexes readily dissociate and free drug molecules remain in equilibrium with the molecules bound within the CD cavity [2].

Here, CDs were used as protecting agent for certain molecules of pharmaceutical interest like viologens, triazinone derivatives and propiconazole. **Viologens** revealed antibacterial efficiency [3] toward Escherichia coli, due to their ability to accomplish DNA strand scission [4]. Methyl viologen (Paraquat) has been extensively used as herbicide and numerous works were done to evaluate its action on the human body because of the severe suspicions about its toxicity against mammals. We proved herein that the toxicity of 4,4'-bipyridyum derivative of viologen can be significantly reduced by including them in tight [2] rotaxane structures alongside β-CD. Among other techniques, the inclusion complex was validated also by cyclic voltammetry showing that the interaction of bipyridil moiety of guest viologen with β-CD host causes pronounced changes in their redox behavior. The lessening of toxicity was also validated by in vivo tests on mice [5].

The chemistry of **1,2,4-triazinone** ring derivatives has attracted increasing attention due to their structures and diverse applications in antibacterials, antidepressants, antiviral drugs, pesticides and herbicide dyes. 1,2,4-Triazin-2-methyl-6-hydroxy-3-thio-5-one (TTZ) is widely used in the production of cephalosporin pharmaceutical intermediates, such as ceftriaxone sodium. The formation of inclusion complexes of TTZ was studied comparatively with α-CD and β-CD. The stable inclusion of TTZ in β-CD is proved by the significant changes of redox activity characteristic for TTZ and good electrochemical stability of the complex. Moreover, the present study demonstrates that β-CD can serve as a carrier system, since the TTZ molecule can be gradually released from the inclusion complex with time [6].

Also, the inclusion complexes of **propiconazole** nitrate (PCZH-NO_3) with substituted β-CD was investigated driven by the fact that fungal infections are an important health issue and azoles, diazoles and triazoles are the most widely used class of antifungal agents. The inclusion complexes formed by PCZH-NO_3 with β-CD was confirmed by NMR experiments and the antifungal properties of the inclusion complex was assessed on 20 Candida spp. clinical isolates (10 Candida albicans + 10 C. glabrata) growing as planktonic phase [7].

References:

[1] Aggarwal, S; Singh, PN; Mishra, B; Studies on solubility and hypoglycemic activity of gliclazide beta-cyclodextrin-hydroxypropylmethylcellulose complexes. *Pharmazie*. **2002**, *57*, 191-193.

[2] Tiwari, G; Tiwari, R; Rai, AK; Cyclodextrins in delivery systems: Applications, *J Pharm Bioallied Sci*, **2010**, *2*, 72–79

[3] Shi, Z; Neoh, KG; Kang, ET; Antibacterial activity of polymeric substrate with surface grafted viologen moieties, *Biomaterials,* **2005**, *26*, 501-508.

[4] Núñez, M. E., Hall, B. D., and Barton, J. K. (1999) Long-range oxidative damage of DNA: effects of distance and sequence. Chem. Biol. 6, 85-97.

[5] Marangoci, N; et al, Low Toxicity β-Cyclodextrin-Caged 4,4'-Bipyridinium-bis(siloxane): Synthesis and Evaluation, *Chem. Res. Toxicol*, **2014**, *27*, 546-557.

[6] Arvinte, A; Marangoci, N; Nicolescu, A; Pinteala, M; Electrochemical evidence for inclusion complexes of thiotriazinone with cyclodextrins, *RSC Advances,* **2016**, *6*, 82817-82823.

[7] Minea, B; Marangoci, Peptanariu, D; Rosca, I; Nastasa, V; Corciova, A; Varganici, C; Nicolescu, A; Fifere, A; Neamtu, A; Mares, M; Barboiu, M; Pinteala, M; Inclusion complexes of propiconazole nitrate with substituted β-cyclodextrins: the synthesis and in silico and in vitro assessment of their antifungal properties. *New J. Chem.,* **2016***, 40,* 1765-1776.

Poster communication 32 - Working Group 2

Structural characterization of C-rich sequence in EGFR promoter region

Camilla Cristofari,[a] Maria Laura Greco,[a] Claudia Sissi[a]

[a] *Dept. of* Pharmaceutical and Pharmacological Sciences, University of Padova, v. Marzolo 5, 35131 Padova, Italy

camilla.cristofari@phd.unipd.it

More than 40% known oncogenes contains C-rich sequences in their promoter region. Their potential folding into non-canonical arrangements, like an i-motif, raised great interest in recent years for therapeutic significance. Indeed, stabilization/induction of this conformation is an interesting strategy to obtain a gene expression modulation and thus a possible new anticancer therapy.

In this work we characterized a 30 bases sequence located upstream the transcriptional start site of EGFR (EGFR-272), that contains 56.6% of cytosine.

By spectroscopic techniques such as circular dichroism and fluorescence melting we confirmed the ability of this sequence to fold into an i-motif in mildly acidic environment (pH 5.0 –6.5). Additionally, we explored the effect of some environmental factors such as the presence of cationic species (K^+, Ag^+ and Ni^{2+}) or cosolvent/dehydrating agents (PEG-300) on the stability of this conformation. Interestingly, our data indicated that the i-motif conformation is stabilized or induced by the presence of Ag^+ or dehydrating agents up to pH values close to the physiological one. Specifically, the presence of PEG-300 at neutral pH, promotes a competition between the i-motif and non-specific DNA-polymer complex.

Finally, we tested three synthetic ligands known for their ability to recognize and interact with G-rich sequences. Our data showed that one bis-phenanthroline analogue alone or coordinated with a metal center is able to recognize the C-rich sequence with high affinity.

Although the validation of the physiological consequences of induction/stabilization is still ongoing, the results here presented show i-motif as a new target for the development of innovative anticancer therapies.

Poster communication 33 - Working Group 2

Dealing with dynamics and disorder of protein kinase inhibitors and their targets as revealed by crystallography and complementary techniques

Richard A. Engh

Dept of Chemistry and Norwegian Structural Biology Center, The Arctic University of Norway, Tromsø, Norway

Richard.Engh@uit.no

The unpredictability of ligand induced structural changes is well known as a major hindrance to structure based drug design. Another challenge is the interpretation of crystal diffraction data, both with respect to the recognition of multiple binding modes, and also with respect to judging the relevance of conformations observed in the crystal to those in the physiological setting. One example from research at the Norwegian Structural Biology Center is the observation by X-ray crystallography of multiple kinase hinge binding modes for benzothiazole based fragments[1], verified to be in dynamic equilibrium by NMR. Another example is the observation of key inhibitor binding interactions with the conserved aromatic side chain of the glycine-rich loop and inhibitors[2]. This loop is particularly flexible (Figure 1), but important inhibitor binding interactions may not be observed in the crystal, depending e.g. on crystal packing conditions[3]. The growth of structural and ligand binding databases promises to compensate for some of this uncertainty. As hundreds of structures of individual targets become available, crystallized with varying conditions, inhibitors, and packing arrangements, representative distributions of target conformations may be observed. These may ultimately be combined with cheminformatics techniques to attempt to link binding data to target conformation[3].

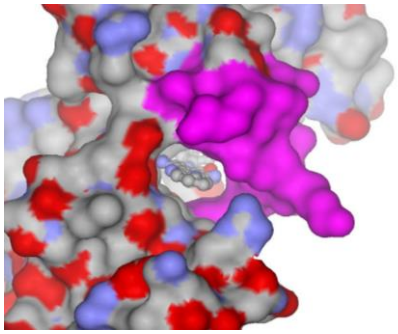


Figure 1: The glycine-rich loop of protein kinases is a major selectivity determinant for inhibitors, but its wide range of conformations pose a challenge both for predicting effects on inhibitor binding energies, and for understanding the relationship between crystal structures and the most physiologically relevant conformations of the protein.

References:

[1] Rothweiler, U.; Stensen, W. Brandsdal, B.O.; Isaksson, J. Leeson, F.A., Engh, R.A; Svendsen, J.S. Probing the ATP-binding pocket of protein kinase DYRK1A with benzothiazole fragment molecules. *J. Med. Chem*, 2016, **DOI:** 10.1021/acs.jmedchem.6b01086.

[2] Lauber, B.S.; Hardegger, L.A.; Asraful, A.K.; Lund, B.A.; Dumele, O.; Harder, M.; Kuhn, B.; Engh, R.A.; Diederich, F. Addressing the glycine-rich loop of protein kinases by a multi-facetted interaction network: Inhibition of PKA and a PKB mimic. *Chemistry*, 2016, 22, 211-221.

[3] Oliveira, T.M.; Ahmad, R; Engh, R.A. VX680 binding in Aurora A: π-π interactions involving the conserved aromatic amino acid of the flexible glycine-rich loop. *J. Phys. Chem. A*. 2011, 115, 3895-3904.

[4] Gani, O.A.; Thakkar, B.; Narayanan, D.; Alam, K.A.; Kyomuhendo, P.; Rothweiler, U.; Tello-Franco, V.; Engh, R.A. Assessing protein kinse target similarity: Comparing sequence, structure, and cheminformatics approaches. *Biochim. Biophys. Acta.* 2015, 1854, 1605-1616.

Poster communication 34 - Working Group 2

DNA binding and biological activity of two novel Cu(II)-quinoxaline complexes

Christiana A. Mitsopoulou,[a] Eutuxia Lioli,[a] Eleni Kolia,[b] Panagiota Markaki[b]

[a] *Inorganic Chemistry Lab, Chemistry Department, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771, Athens, Greece*

[b] *Food Chemistry Lab., Chemistry Department, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15784, Athens, Greece*

cmitsop@chem.uoa.gr

Nowadays, the increasing number of cancers cases leads to the demand of effective treatments without side effects. Over the last decades, intense research has focused in the effects of small compounds that noncovalently bind to nucleic acids. Accordingly, DNA binding compounds have potential applications as anti-cancer and anti-fungal agents. In all these compounds the role of the metal ion is very important since it can easily modify not only the binding properties of an organic molecule but also its photospectroscopic properties.

In this context, herein is presented the synthesis of two novel copper (II) complexes carrying 2-(2'-pyridyl quinoxaline) ligands. Binding of the complexes with Calf thymus DNA (CT-DNA) has been investigated by different spectroscopic and biological methods both in dark and after illumination with $\lambda > 400$nm.[1,2]

In addition to these experiments, a study was carried out investigating the anticancer as well as the anti-fungal efficacy of the aforementioned two complexes against Aspergillus parasiticus in different concentrations.

References:

[1]Chen, G. J.; Qiao, X.; Qiao, P.-Q.; Xu, G.-J.; Xu, J-Y.; Tian, J. –L.; Gu, W.; Liu, X.; Yan, S.-P. Synthesis, DNA binding, photo-induced DNA cleavage, cytotoxicity and apoptosis studies of copper(II) complexes *J. Inorg. Biochem.,* **2011**, 105, 119-126.

[2]Kaplanis, M.; Stamatakis, G.; Papakonstantinou, V.D.; Paravatou-Petsotas, M.; Demopoulos, C.A.; Mitsopoulou, C.A., Re(I) tricarbonyl complex of 1,10-phenanthroline-5,6-dione: DNA binding, cytotoxicity, anti-inflammatory and anti-coagulant effects towards Platelet Activating Factor. *J. Inorg. Biochem*., **2014**, *135*, 1-9.

Poster communication 35 - Working Group 2

New drug candidates targeting hydrogen sulfide

Gunay Yetik-Anacak,[a] Mehmet Vehbi Dereli,[a] Ozge Ozzayim,[a] Gulnur Sevin[a]

[a] *Dept of Pharmacology, Faculty of Pharmacy, Ege University, 35100 Bornova, Izmir, Turkey*

gunayyetik@gmail.com

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

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

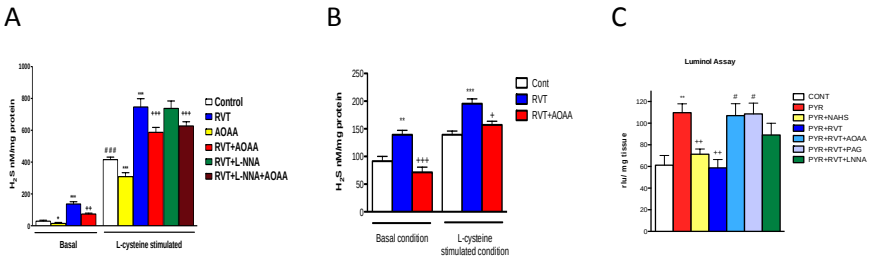


Figure 1: Figure 1: The effect of RVT on H_2S formation in A) MCC and B) mouse aorta and C) reactive oxygen species. RVT significantly increased H_2S level in basal and L-cysteine stimulated conditions and AOAA prevented this augmentation. *p<0.05,**p<0.01,***p<0.001 compared to control, +p<0.05, ++p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

RVT stimulated both basal and L-cysteine-induced H_2S formation in both aorta and penile tissue. H_2S inhibitor AOAA or PAG inhibited the augmented H_2S formation and endogenous H_2S-dependent relaxation, suggesting the role of H_2S in vasorelaxant effect of RVT, (Figure 1). RVT relaxed MCC dose dependently (96.90± 4.26 vs 123.4±3.755) and this relaxation was inhibited by AOAA or AOAA+PAG (123.4±3.755 vs 96.85 ± 3.474 and 96.90 ±4.260, p<0.01, n=12, 8 and 5 respectively) but not by L-NNA[5]. RVT inhibited pyrogallol-induced radical generation in mouse aorta (p<0.05, n=5). AOAA significantly reversed the inhibitor effect of RVT on pyrogallol-induced ROS formation (p<0.05, n=5).

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

Acknowledgements: We thank for the financial supports by Turkish Scientific Research Council TUBITAK for the grant #114s448 and the COST action CA15135.

References:

1. Yang, G. and R. Wang, Handb Exp Pharmacol, **2015**. **230**: p. 85-110.

2. Wallace, J.L. and R. Wang, Nat Rev Drug Discov, **2015**. **14**(5): p. 329-45.

3. Boydens, C., et al., J Sex Med, **2015. 12**(2): p. 303-12.

4. Das, S. and D.K. Das, Recent Pat Cardiovasc Drug Discov, **2007. 2**(2): p. 133-8.

5. Yetik-Anacak, G., et al., J Sex Med, **2015. 12**(10): p. 2004-12.

Poster communication 36 - Working Group 3

SAR-DB: A Structure-Activity Database for Virtual Screening

José M. Padrón,[a] Miguel X. Fernandes[a]

[a] *BioLab, Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, C/ Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain*

jmpadron@ull.es

Within our program directed at the discovery of new antitumor agents, we have implemented a database, SAR-DB, which holds the chemical structure and all the results arising from the screening experiments performed in our research group. SAR-DB allows the fast retrieval of all information concerning the biological assay of any given compound from the chemical library. At present SAR-DB contains information for more than 4,000 chemical compounds from diverse origin: natural products, semisynthetic and synthetic, organic and inorganic small molecules. The compounds have been provided not only by the chemistry groups from the University of La Laguna, but from long-term establish collaborations with other national and international (non-medicinal) chemistry groups.

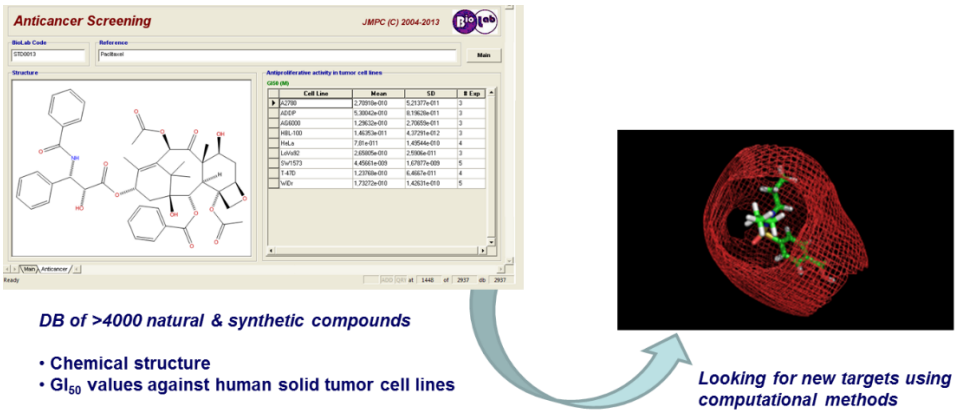


Figure 1: Structure-Activity DB for Virtual Screening.

From a small subset of SAR-DB, we have described recently the benefit of artificial neural networks to establishing relationships between compounds' structure and their antiproliferative activity.[1,2] The ultimate goal is to establish a statistical model(s) that will allow the prediction of activity of novel compounds before laborious and expensive synthesis and experimental testing. Relation between descriptors (independent variables) and activity (dependent variable) can be retrieved using linear and nonlinear methods, and accepted models must allow an easy translation of its parameters into synthetic chemical features.

References:

[1] Sousa, I. J.; Padrón, J. M.; Fernandes, M. X. Generation of artificial neural networks models in anticancer study. *Neural Comput. Applic.*, **2013**, *23*, 577-582.

[2] Silveira-Dorta, G.; Sousa, I. J.; Fernandes, M. X.; Martín, V. S.; Padrón, J. M. Synthesis and identification of unprecedented selective inhibitors of CK1ε. *Eur. J. Med. Chem.*, **2015**, *96*, 308-317.

Poster communication 37 - Working Group 3

Conf-VLKA: A structure-based revisitation of the Virtual Lock-and-Key Approach

Ugo Perricone,[a] Marco Tutone,[a] Anna Maria Almerico[a]

[a] *Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università di Palermo, Via Archirafi 28, Palermo-Italy.*

ugo.perricone@unipa.it

In a previous work, we developed the *in house* Virtual Lock-and-Key Approach (VLKA) to evaluate target assignment starting from molecular descriptors calculated on known inhibitors used as information source[1]. This protocol was able to predict the correct biological target for the whole dataset with a good degree of reliability (80%), and proved experimentally useful for the target fishing of unknown compounds. In this paper, we tried to remodel the previous *in house* developed VLKA in a more sophisticated one in order to evaluate the influence of 3D conformation of ligands on the accuracy of the prediction.

In the attempt to give more accuracy to VLKA, we decided to use the same previous algorithm of scoring and ranking but this time combining it with a structure-based approach as docking. For this reason, we retrieved from the RCSB Protein Data Bank (PDB), the available 3D structures of the biological targets included into the previous work, and we used them to calculate the top-ranked poses of the 7352 dataset compounds in the VLKA biological targets. Docking protocol have been used to retrieve docking scores, first, and, from the best poses for each molecule, to re-calculate 3D-descriptors (Conf-VLKA), which consist of 142 out of 173 descriptors originally used in the VLKA. While the use of the simple docking scores proved to be inadequate to improve compounds classification, the Conf-VLKA represented an improvement (86%) of the original VLKA, especially for targets whose ligands present a high degree of branching.

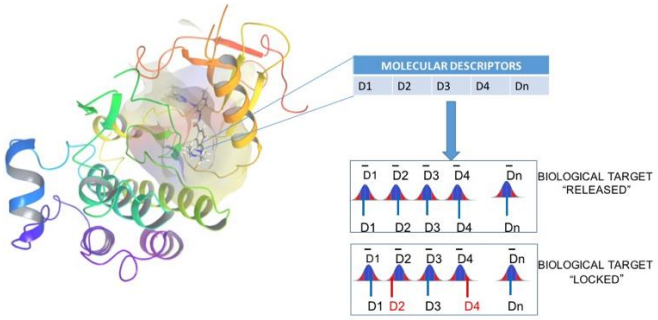


Figure 1: Graphical representation of the conf-VLKA.

References:

[1] Lauria A.; Tutone M.; Almerico AM. Virtual Lock-And-Key Approach: The In Silico Revival of Fischer Model By Means Of Molecular Descriptors. *Eur J Med Chem,* **2011**, 46:4274-428.

Poster communication 38 - Working Group 4

Novel drug discovery strategy against alcoholism, in silico approach to the protein tyrosine phosphatase receptor Z1 (PTPRZ1)

Bruno Di Geronimo, Claire Coderch, Ana Ramos and Beatriz de Pascual-Teresa

Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU San Pablo, 28668 Boadilla del Monte, Madrid, Spain.

bru.digeronimo.ce@ceindo.ceu.es

A new approach for preventing alcoholism needs to be considered as it is deemed the third highest death risk factor by the World Health Organization (WHO), not only due to the harmful effects of alcohol on health, but also to the social and economic burden that it causes in many countries.[1]

Recently, experiments have proven that the over expression of pleiotrophin (PTN) impairs the addictive effects of ethanol in mice.[2] The PTN pathway interacts with the extracellular domain of Protein Tyrosine Phosphatase Receptor Z1 (PTPRZ1) by blocking its phosphatase activity and increasing phosphorylation levels of other protein substrates. Therefore, the main goal of our work is to mimic the effect of PTN through the inhibition of PTPRZ1 with small molecules as a new strategy for the treatment of alcoholism.

As a starting point, the design of new analogues was based on the structure of two recently reported compounds (4-trifluoromethylsulphonylbenzyl and 4-trifluoromethylsulphonylphenyl) that present a certain degree of activity and selectivity for PTPRZ1.[3] Moreover, we aim to improve the pharmacokinetic properties of all the analogues in order to favor the crossing of the Blood Brain Barrier (BBB).

The proposed binding-modes and binding affinities were assessed by means of molecular dynamics studies (including MD-ISMSA) providing results that were in agreement with recent in vivo studies. The crystal structure and several homology models of PTPRZ1 that map the conformational flexibility of the catalytically essential WPD-loop, were used as targets in order to search for a plausible binding site for the new analogues. The proposed binding-modes and binding affinities were assessed by means of molecular dynamics studies providing results that were in agreement with recent *in vivo* studies.

References:

(1) Based on the Global status report on alcohol and health 2014.

(2) Vicente-Rodríguez, M.; Pérez-García, C.; Ferrer-Alcón, M.; Uribarri, M.; Sánchez-Alonso, M. G.; Ramos, M. P.; Herradon, G. Pleiotrophin Differentially Regulates the Rewarding and Sedative Effects of Ethanol. *J.Neurochem.* **2014**, *131* (5), 688–695.

(3) Huang, P.; Ramphal, J.; Wei, J.; Liang, C.; Jallal, B. Structure-Based Design and Discovery of Novel Inhibitors of Protein Tyrosine Phosphatases. *Bioorganic & Medicinal Chemistry.* **2003.**

Acknowledgments. This work is supported by Ministerio de Sanidad (MSSSI), Plan Nacional Sobre Drogas (PNSD2015I001). We thank Fundación Universitaria San Pablo CEU for a fellowship to BdiG and to Ministerio de Educacion, Ciencia y Desarrollo (MECD) for a FPU15/02857 scholarship.

Poster communication 39 - Working Group 4

Combination of pharmacophore and docking methods: a success story on the repositioning approach for the discovery of EBOV VP35 inhibitors

Simona Distinto,[a] Gian Luca Daino,[a] Aldo Frau,[a] Angela Corona,[a] Giulia Bianco,[a] St. Patrick Reid,[b] Rita Meleddu,[a] Claudia Melis,[a] Stefano Alcaro,[c] Sina Bavari,[b] Elias Maccioni,[a] Enzo Tramontano[a]

[a]*Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy.*
[b]*USAMRIID, Frederick, MD, USA.*
[c] *Dipartimento di Scienze della Salute, Università "Magna Graecia" di Catanzaro.*

s.distinto@unica.it

Ebola virus (EBOV) is the etiological agent of Ebola Virus Disease (EVD). The high EVD lethality has been attributed to the EBOV ability to efficiently bypass the host innate antiviral response that begins with the recognition of the viral dsRNA by the cytoplasmic RIG-I receptor that, in turn, induces type I interferon alpha/beta (IFN α/β) production. EBOV VP35 is a multifunctional viral protein that mimics RIG-I recognition of the 5'-triphosphorylated dsRNA and hides to the cellular receptor the viral dsRNA.[1]

Given the specific dsRNA EBOV VP35 recognition, dsRNA binding site is an appealing target for drug design.[2] Hence, after the identification of the most critical residues involved in VP35 dsRNA binding, we built a pharmacophore model with LigandScout software.[3] The model has been used as a filter to screen a database of around 2500 drugs, further analyzed by consensus docking with GlideXP,[4] QMPLD[5] and Autodock.[6] This approach is known as repositioning approach, which is the discovery of new indications for approved or failed drug.[7]

Among the compounds, 10 hits were selected and tested for the ability to inhibit the VP35 in biochemical assay, cellular assay and on a reporter gene assay measuring the IFN-antagonist ability of VP35 in cell cultures. Preliminary studies indicated that among them 2 compounds were able to block the dsRNA binding in the μM range.

References:

1. Leung, D. W.; Prins, K. C.; Basler, C. F.; Amarasinghe, G. K. Ebolavirus VP35 is a multifunctional virulence factor. *Virulence* **2010,** 1, 526-31.

2. Zinzula, L.; Tramontano, E. Strategies of highly pathogenic RNA viruses to block dsRNA detection by RIG-I-like receptors: hide, mask, hit. *Antiviral Res.* **2013,** 100, 615-35.

3. InteLigand Software GmbH. *LigandScout 3.0*, Maria Enzersdorf, Austria.

4. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein−Ligand Complexes. *J. Med. Chem.* **2006,** 49, 6177-6196.

5. Schrödinger LLC. *QMPolarized protocol*, 2012; New York, NY, USA.

6. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009,** 30, 2785-2791.

7. Ashburn, T. T.; Thor, K. B. Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.* **2004,** 3, 673-683.

Poster communication 40 - Working Group 4

Molecular docking studies of novel 9-aminoacridine derivatives with potential multi-target-based antiproliferative activity

Vladimir Dobričić,[a] Jelena Pantić,[a,b] Mara Aleksić,[b] Jasmina Brborić,[a] Sote Vladimirov,[a] Olivera Čudina[a]

[a]*Dept of Pharmaceutical Chemistry, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia*

[b]*Dept of Physical Chemistry and Instrumental Methods, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia*

vladimir@pharmacy.bg.ac.rs

Antiproliferative activity of acridine derivatives is mainly result of the intercalation into DNA and subsequent inhibition of DNA related enzymes, such as topoisomerases and telomerase. Recent studies showed potential of acridine derivatives to inhibit enzymes involved in tumor cell growth, such as Src, MEK and VEGFR-2 kinases.[1,2] The aim of this study was design of novel 9-aminoacridine derivatives with potential antiproliferative activity based on multi-target action – DNA intercalation, inhibition of Src, MEK or VEGFR-2 kinases. Chemical structures of designed derivatives are presented in Figure 1. Interactions of these compounds with selected targets (DNA, src, MEK and VEGFR-2) were analyzed in AutoDock Vina program and compared to the interactions of corresponding co-crystallized ligands (amsacrine, H8H, EUI and LIF, respectively).

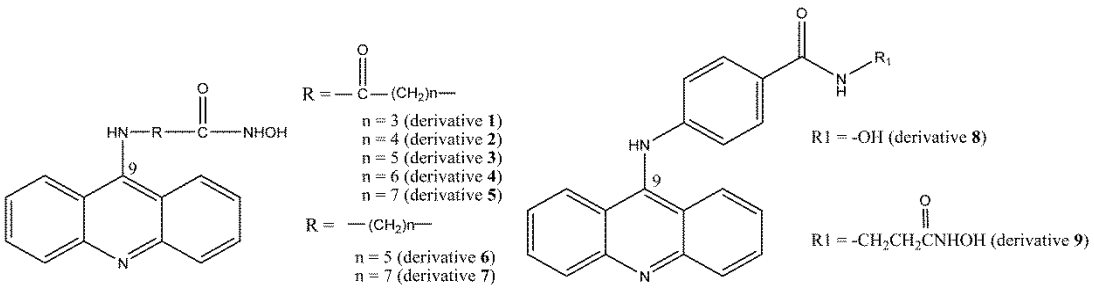


Figure 1: Chemical structures of designed derivatives.

All tested compounds bind to DNA similarly to amsacrine. Derivatives with binding energies similar to corresponding co-crystallized ligands which form some of the key binding interactions with MEK were **3**, **4**, **5**, **6**, **7** and **9**, whereas with src were **1**, **3**, **7** and **8**. Derivatives **3**, **4**, **5**, **8** and **9** form some of the key binding interactions with VEGFR-2 but their binding energies were significantly higher in comparison to LIF.

References:

[1]Luan, X.; Gao, C.; Zhang, N.; Chen, Y.; Sun, Q.; Tan, C.; Liu, H.; Jin, Y.; Jiang, Y. Exploration of acridine scaffold as a potentially interesting scaffold for discovering novel multi-target VEGFR-2 and Src kinase inhibitors. *Bioorgan. Med. Chem*, **2011**, *19*, 3312-3319.

[2]Cui, Z.; Li, X.; Li, L.; Zhang B.; Gao, C.; Chen Y.; Tan C.; Liu H.; Xie W.; Yang. T.; Jiang Y. Design, synthesis and evaluation of acridine derivatives as multi-target Src and MEK kinase inhibitors for anti-tumor treatment. *Bioorgan. Med. Chem*, **2016**, *24*, 261-269.

Poster communication 41 - Working Group 4

Computational Approaches for the Discovery of Human Proteasome Inhibitors

R. A. Guedes,[a] J. A. R. Salvador,[b,c] R. Gavioli,[d] R. C. Guedes[a]

[a]*iMed.ULisboa – Research Institute of* Medicines, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; [b]Laboratório de Química Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra; [c]Center for Neurosciences and Cell Biology, Universidade de Coimbra; [d]Department of Life Sciences and Biotechnology , University of Ferrara , Ferrara , Italy.

rguedes@ff.ulisboa.pt

Proteasome emerged as an important target in recent pharmacological research due to its pivotal role in degrading proteins in the cytoplasm and nucleus of eukaryotic cells, regulating a wide variety of cellular pathways, including cell growth and proliferation, apoptosis, DNA repair, transcription, immune response, and signaling processes. The last two decades witnessed intensive efforts to discover 20S proteasome inhibitors with significant chemical diversity and efficacy. To date, the US FDA approved to market three proteasome inhibitors: bortezomib, carfilzomib, and ixazomib. However new, safer and more efficient drugs are still required. Computer-aided drug discovery has long being used in drug discovery campaigns targeting the human proteasome. The aim of this work is to illustrate selected *in silico* methods like homology modeling, molecular docking, pharmacophore modeling, virtual screening, and combined methods that have been used in proteasome inhibitors discovery. Applications of these methods to proteasome inhibitors discovery will also be presented and discussed to raise improvements in this particular field.

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

References:

[1] Guedes, R. A.; Serra, P.; Salvador, J. A. R.; Guedes, R. C.. Computational Approaches for the Discovery of Human Proteasome Inhibitors: an overview. *Molecules*, **2016**, 21, 927.

Poster communication 42 - Working Group 4

Multi-Approach Virtual Screening. Complex solution in the design of multitarget directed ligands

Jakub Jończyk,[a] Marek Bajda,[a] Barbara Malawska[a]

[a]*Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

jakub.jonczyk@doctoral.uj.edu.pl

The development of system biology resulted in expanding our knowledge about complexity of disease processes. Many failures of drug candidates in clinical trials have shown that aiming single biological target may be ineffective. It is a reason why multitarget directed ligand (MTDL) discovery is one of the hottest topics especially in the case of complex diseases such as cancer or Alzheimer's disease[1]. In the rational design of MTDLs a combination of pharmacophores is used. Pharmacophore combination is a knowledge-based approach which searches a way to connect two or more molecular frameworks into a new single multi-targeted compound through connecting of different pharmacophores by linker or directly. However, this process leads to the compounds with relatively high molecular weight and poor physicochemical parameters. Another way is to merge pharmacophores enabling synthesis of compounds with low molecular weight. However, this is a way that causes a lot of problems during development of the substance[2]. Computer-aided design methods allow to search very large databases and check the multiple hypotheses but fast screening methods are usually burdened with large errors in the prediction of activity compounds. As the solution to these problems multi-level screening approach can be used. It is based on the structure of known ligands and biological targets. In the presented study we applied the multi-approach virtual screening to combine activity of A_{2A} adenosine receptor antagonist and BACE1 inhibitor. Selective A_{2A} adenosine receptor antagonists proved capability of preventing neurotoxicity induced by Aβ peptide while β-secretase inhibitors can decrease production of neurotoxic peptide in Alzheimer's brain. Both effects together can stop the progression of degenerative processes by lowering Aβ levels and preventing neurons from amyloid deposits which have already arisen[3,4]. Our screening began with LiSiCA program, using the Tanimoto coefficients for comparing large compound libraries with reference ligands. Ligands with the highest similarity to A_{2A} adenosine receptor antagonists and BACE1 inhibitors has been docked with the GOLD program to crystal structures of BACE1 (2QU2, 3KNO) and A_{2A} (3UZC, 5K2A). Top rated by the ChemPLP scoring function ligands have been docked using the Glide with XP algorithm. The compounds with the highest values of docking scores in both docking programs were selected for further study.

References:

[1] Bolognesi ML.; Cavalli A. Multitarget Drug Discovery and Polypharmacology. *ChemMedChem*, **2016**, *11*, 1190-1192.

[2] Prati F.; Cavalli A.; Bolognesi ML. Navigating the Chemical Space of Multitarget-Directed Ligands: From Hybrids to Fragments in Alzheimer's Disease. *Molecules*, **2016**, *21*, 466.

[3] Takahashi RN.; Pamplona FA.; Prediger RD. Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. *Frontiers in Bioscience*, **2008**, *13*, 2614-2632.

[4] Yan R. Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. *Transl Neurodegener*. **2016**, *5*, 13.

Poster communication 43 - Working Group 4

Identification of anti-obesity side effects of FDA-approved drugs through computer-aided repurposing techniques

Isabella Romeo, Giosuè Costa, Anna Artese, Stefano Alcaro

Dept of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Catanzaro, Italy.

isabella.romeo@unicz.it

Previous studies evidenced that Carbonic Anhydrase inhibitors (CAIs) are emerging as anti-obesity drugs[1]. As reported in literature, the inhibition of such CAs by sulfonamides, the main class of CAIs in clinical use, decreases lipogenesis in adipocytes in cell culture[2]. For example, topiramate, an antiepiletic drug, was associated to a loss of body weight observed in obese patients, although no pharmacological explanation of this phenomenon has been provided. Moreover, further studies demonstrated that topiramate was a very potent inhibitor of several CA isozymes, revealing the molecular interactions responsible for the high affinity of this compound *versus* the enzyme active site and rationalizing its use to control weight loss and obesity. These observations were useful to explain the side effects observed in obese epileptic patients treated with this drug[3,4]. On the basis of this data, we performed a virtual screening on the DrugBank database[5,6], which compiles FDA-approved drugs, *versus* the mitochondrial isoform VA of the CAs family. We used the crystallographic X-ray model of murine carbonic anhydrase deposited in the Protein Data Bank[7] with the code 1DMY, in order to generate the starting structure with the human isoform VA sequence, by using UniProt database[8]. We built and refined the enzyme adopting the Prime homology modeling program[9]. We submitted molecular dynamics simulations using Desmond package[10] and, after clustering the trajectory, we minimized all the obtained complexes. The Enhrichment factor analysis was adopted to select the best model for the virtual screening, carried out by means of Glide[11] SP protocol.

Several drugs, such as Lenvatinib, Pazopanib, Rufinamide, Pralatrexate, some cephalosporins and antiviral inhibitors, demonstrated a good binding affinity towards the CAs, thus suggesting their implications in the unexplained weight loss, in agreement with the literature data[12,13] (**Figure 1**).

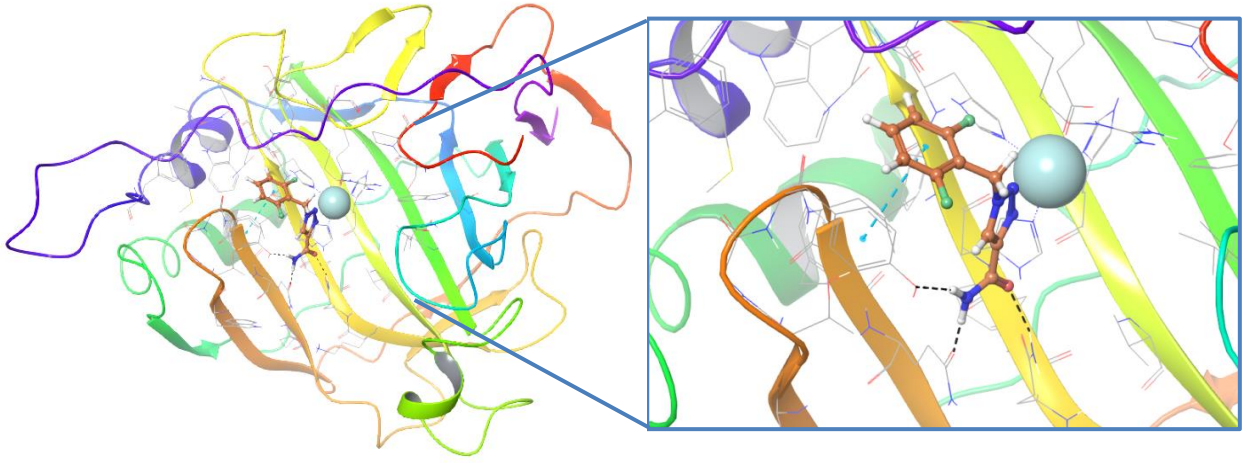


Figure 1: 3D representation of the isoform VA CAs in complex with the best pose of Rufinamide.

The obtained results highlighted the potential of computer-aided drug repositioning and the *in silico* prediction of side effects in order to search new therapeutic indications, as the inhibition of the lipogenesis, for already known drugs and to exploit an antiobesity beneficial effect in the compromised patients.

References:

[1] Supuran, CT.; Scozzafava, A.; Casini, A. Carbonic anhydrase inhibitors. *Med Res Rev*, **2003**, *23*, 146-189.

[2] Spencer, IM.; Hargreaves, I.; Chegwidden, WR. Effect of the carbonic anhydrase inhibitor acetazolamide on lipid synthesis in the locust. *Biochem Soc Trans*, **1988**, *16,* 973-974.

[3] Supuran, CT.; Di Fiore, A.; De Simone, G. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. *Expert Opin Emerg Drugs*, **2008**, *13*, 383-392.

[4] Casini, A.; Antel, J.; Abbate, F. Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfamates/sulfamides with isozymes I, II and IV. *Bioorg Med Chem Lett*, **2003**, *13*, 841-845.

[5] Knox, C.; Law, V.; Jewison, T. DrugBank 3.0: a comprehensive resource for 'Omics' research on drugs. *Nucleic Acids Res*, **2011**, *39,* 1035-1041.

[6] Wishart, DS.; Knox, C.; Guoetal, AC. DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. *Nucleic Acids Res*, **2006**, *34*, 668-672.

[7] http://www.rcsb.org/pdb

[8] The UniProt Consortium. UniProt: a hub for protein information. *Nucleic Acids Res*, **2015**, *43*, 204-212.

[9] Prime, version 4.4, Schrödinger, LLC, New York, NY, **2016**.

[10] Desmond Molecular Dynamics System, version 3.4, D. E. Shaw Research, New York, NY, **2013.**

[11] Glide, version 6.2, Schrödinger, LLC, New York, NY, **2014**.

[12] Mourand, I.; Crespel, A.; Gelisse, P. Dramatic weight loss with rufinamide. *Epilepsia,* **2013**, *54*, 5-8.

[13] Van der Graaf, WTA.; Blay, JY.; Chawla, SP.; Kim, DW. et al. Pazopanib for metastatic soft-tissue sarcome (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*, **2012**, *379*, 1879-1886.

Poster communication 44 - Working Group 4

Application of 3D-QSAR and virtual screening methods for design of novel antidepressants affecting serotonin transporters and histamine H_3 receptors

Jelica Vucicevic,[a] Nemanja Djokovic,[a] Slavica Filipic,[a] Katarina Nikolic,[a] Danica Agbaba[a]

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

jelicav@pharmacy.bg.ac.rs

Depression is the most common psychiatric disease. There are indications that dual histamine H_3 antagonsts with selective serotonin reuptake inhibition activity could be promising novel class of more effective antidepressants.[1] The aim of this *in silico* study was to identify the novel dual acting antidepressants with suitable pharmacokinetic properties, by using computer-aided drug discovery tools. Two 3D-QSAR models were developed, one for each of target sites, 3D-structure of pharmacophores were defined and used to design novel dual ligands (**Fig. 1**). The 20 novel ligands with optimal activities and estimated pharmacokinetic profiles were selected.

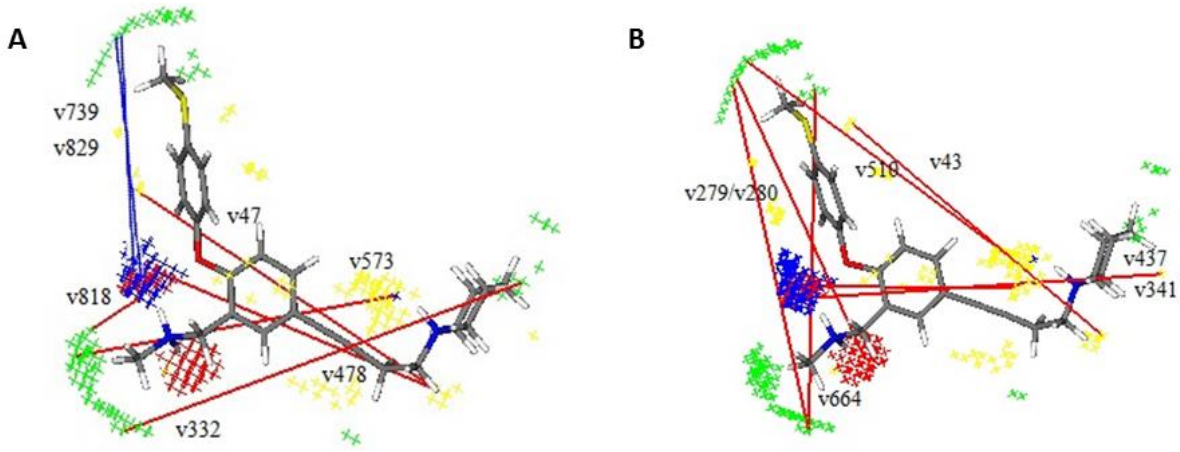


Figure 1: Pharmacophore models of H_3R antagonist (**A**) and serotonin reuptake inhibitors (**B**)

In the second part of this study, virtual screening on ZINC database of commercially-available compounds has been performed. Activity prediction by 3D-QSAR models has showed good correlation with screening results. Novel compounds have been pointed out as good starting point for development of completely new classes of dual antidepressants.

References:

[1] Anighoro, A.; Bajorath, J.; Rastelli G. Polypharmacology: challenges and opportunities in drug discovery. *J Med Chem.* 2014, 57(19): 7874-87.

This project was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract #172033.

Poster communication 45 - Working Group 4

The Big Data Challenge in Drug Design: Data Visualization

Abraham Yosipof,[a] and Hanoch Senderowitz[b]

[a] *Dept of Business Administration, College of Law & Business, Ramat-Gan, Israel*

[b] *Dept of Chemistry, Bar-Ilan University , 5290002, Ramat-Gan, Israel*

avi.yosipof@gmail.com

Today, data bases become increasingly large as well as increasingly complex. Visualization of high dimensional data is an important problem in many different domains and especially in drug design. Visualization of chemical data and a good representation of the chemical space is useful in many chemoinformatic and drug design applications including the selection of compounds to synthesis, the selection of compounds for biological evaluation, selection of subsets for the design of information-rich compound libraries, and even for the development of reliable QSAR models. The main problem of visualization of high dimensional data concerns the data representation in 2D or 3D with minimal loss of information. Furthermore, data visualization techniques are using dimensionality reduction methods in order to get a 2D or 3D representation of the data. The dimensionality reduction aim is to preserve as much of the significant structure of the high-dimensional data as possible in the low-dimensional map.

Here we present the first implementation of t-Distributed Stochastic Neighbor Embedding (t-SNE)[1] method for the visualization and the representation of the chemical space. In order to get a good representation of the chemical space we coupled the t-SNE algorithm with an optimization engine for feature selection. To test the algorithm, we used two data bases (1) The Comprehensive Medicinal Chemistry (CMC) database**.** This database contains 4,855 pharmaceutical compounds classified into 105 different biological indications, were each compound is characterized by 39 calculated descriptors. (2) The Bitter database. This database contains 1527 non-bitter compounds and 547 bitter compounds, were each compound is characterized by 19 calculated descriptors.

The newly t-SNE optimization algorithm produce a 2D representations of the data bases. The 2D representations were evaluated by standard parameters such as the trustworthiness of the low-dimensional embedding. The algorithm captured much of the local information of the high-dimensional data very well, while also revealing global information such as the chemical space, which clearly shows visual separation of the data to the correct clusters.

References:

[1] Maaten, L. v. d.; Hinton, G., Visualizing data using t-SNE. *Journal of Machine Learning Research* **2008**, 9, 2579-2605.

Author index

Legend

PL = plenary lectures; SC = short communications; P = poster communications