# Second WG Meeting
# CA15135

## BOOK OF THE ABSTRACTS

## COST ACTION CA15135

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


**Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG) - Universidad de La Laguna, Tenerife (Spain), March 15-16 2018**

**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. Started in 2016, our COST Action has now almost arrived at the "turning point". As planned in the Memoradum of Understanding document two annual meetings (Lugano and Porto), two training schools (Vienna and Siena) and two WG meetings (Budapest and Tenerife) have been organized in the first two grant years. The Chemotheca, a new virtual tool to speed up discovery of novel MTDL agents aggregating the research teams belonging to 33 different pan-European countries, has been developed and launched within all participants. Several STSM programs have been supported and many European connections have activated or potentiated. Dissemination activities were activated too.

The WG meeting in Tenerife is the appropriate occasion to check the status of the work done, in order to properly fulfill all the issues of the COST Action second progress review to be presented with few weeks. Also in this case experts of each WG were invited to give plenary lectures. A large space was reserved to short communications, given by MC board components as well as by young investigators, belonging to research institutions located in different places of the MuTaLig network, according to the gender balance. A final round table and the CORE meeting will complete the two intense days of the WG meeting.

As Chair of this COST Action, I want to express my gratitude especially to the local organizer and LOS (Prof. José Padron, MC substitute for Spain), to his local team, to the Grant Holder from University of Porto (Prof. Fernanda Borges and Dr. Joana Maria Neves Moreira Abrantes) and to the COST Association (Dr. Lucia Forzi, Science Officer and Dr. Svetlana Voinova, Administrative Officer) for their efforts in the meeting organization. A special thank is also due to the young investigator Dr. Carmine Talarico (Università "Magna Græcia" di Catanzaro, Italy) for the support in the organization of this abstract book.

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

Stefano Alcaro
Università "Magna Græcia" di Catanzaro (Italy)
Chair of CA15135 COST Action
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## Local Organizing Committee

José M. Padrón

Miguel X. Fernandes

Irene Lagunes

Amina Moutayakine

Alexis Galán Rodríguez

## Acknowledgments

# PROGRAM

## Thursday March 15th 2018

8.30 Registration

9.00 **Introduction to the MuTaLig COST Action 2nd WG meeting**
Manuel NORTE (IUBO AG Director) - Universidad de La Laguna, Tenerife (Spain)
Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)
José M. PADRON (MC substitute for Spain and local organizer) - Universidad de La Laguna, Tenerife (Spain)

**Session I "Reports by WG leaders on the status of WG activities"**
**Moderator: Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)**

9.15 **WG1 Report**
Danijel KIKELJ (WG1 leader) - University of Ljubljana (Slovenia)

9.30 **WG2 Report**
Eugenio GAUDIO (WG2 leader) - Oncology Research Institute, Bellinzona (Switzerland)

9:45 **WG3 Report**
Sharon BRYANT (WG3 leader) - Inte:Ligand GmbH, Vienna (Austria)

10.00 **WG 4 Report**
Hanoch SENDEROWITZ (WG4 leader) - Bar-Ilan University, Ramat-Gan (Israel)

10.15 *Coffee break*

**Session II "Towards new chemical entities within WG1"**
**Moderator: Ivo Grabchev (MC member Bulgaria)**

10.45 PL1 **New molecular entities as inhibitors of HBV and (re)emerging virus replication**
Maria Josè CAMARASA (invited speaker) - Instituto de Química Médica (IQM, CSIC), Madrid (Spain)

11.15 OC1 **Novel indanone derivatives as MAO B/H3R dual targeting ligands for treatment of Parkinson's disease**
Holger STARK (MC member DE) - University Duesseldorf (Germany)

11.30 OC2 **Biological activity of novel benzylidene-based (1,3-selenazol-2-yl)hydrazones**
Nenad FILIPOVIĆ - University of Belgrade (Serbia)

11.45 OC3 **The utility of the xanthine scaffold for developing MAO-B inhibitors and antioxidants**
David SYNAK- Jagiellonian University Medical College, Kraków (Poland)

12.00 OC4 **Chalcogen-based privileged structures in the design of new multitarget drugs**
Óscar LÓPEZ - University of Sevilla (Spain)

12.15 OC5 **Development of multi-target agents based on hydroxybenzoic acid scaffold endowed with mitochondriotropic antioxidant and cholinesterase inhibitory activities**
Catarina OLIVEIRA - Universidade do Porto (Portugal)

12.30 OC6 **Naphthalimide Molecular Logic Gates as Fluorescence Cellular Imaging and Anticancer Agents**
David MAGRI (MC member ML) - University of Malta (Malta)

12.45 OC7 **Multi-target paradigm for innovative ligand identification in anticancer drug discovery process**
Christian MULLER - Université de Strasbourg, Illkirch (France)

13.00 OC8 **Symmetry of Ligands: Matriptase-2 Inhibitors as a Case Study in Drug Design**
Michael GÜTSCHOW - University of Bonn (Germany)

13.15 *Lunch*

**Session III "Selection of biological targets and assessment of biological data within WG2"**
**Moderator: Maria Laura Bolognesi (CA15135 STSM coordinator) "Alma Mater" Università di Bologna (Italy)**

14.15 PL2 **MicroRNAs in Oncogenesis: Leading or Passenger? It doesn't matter!**
Rami AQEILAN (invited speaker) - The Hebrew University Faculty of Medicine, Jerusalem (Israel)

14.45 OC9 **The propargylamine warhead in multi-target compounds for inactivation of MAO**
Rona RAMSAY (MC member UK) - University of St Andrews (UK)

15.00 OC10 **Hydrogen sulfide: a candidate multitarget ligand for cardiovascular diseases**
Gunay YETIK ANACAK (MC member TK) - Ege University, Izmir (Turkey)

15.15 OC11 **Combinatorial screening of the pan-PI3K inhibitor Copanlisib in T cell lymphomas**
Eugenio GAUDIO (MC member CH, WG2 leader) - Institute of Oncology Research, Bellinzona (CH)

15.30 OC12 **Metformin interference with survival signaling in DLBCL**
Mirza SULJAGIC (MC member BA) - International University of Sarajevo (Bosnia and Herzegovina)

15.45 OC13 **Mitochondriotropic antioxidants based on cinnamic acid increase cellular stress responses in HepG2 cells**
Ricardo AMORIM - Universidade do Porto (Portugal)

16.00 OC14 **Unbiased assessment of direct or indirect targets of any bioactive compound on a proteome-wide scale**
Susana CRISTOBAL (MC member SE) - Linköping University (Sweden)

16.15 OC15 **Histamine $H_4$ receptors a new therapeutic target in postinfectious cardiomyopathy?**
Wieslawa Agnieszka FOGEL (MC member PL) - University of Lodz (Poland)

16.30 *Coffee break and Poster session*

**Session IV "Development of chemical databases within WG3"**
**Moderator: Sharon Bryant (MC member Austria, WG3 leader)**

17.00 PL3 **Structure of human asparagine synthetase informs the development of new reagents for probing the metabolism of sarcoma cells**
Nigel RICHARDS (invited speaker) - University of Cardiff (UK)

17.30 OC16 **Molecular modeling of novel MAO-B/AChE dual inhibitors as potential anti-Parkinson agents**
Donatella BAGETTA - Università "Magna Græcia" di Catanzaro (Italy)

17.45 OC17 **Tau-directed polypharmacological approach in Alzheimer's disease: development of a small library of thiazolidinediones**
Annachiara GANDINI - "Alma Mater" Università di Bologna (Italy)

18.00 OC18 **Development of a plant genetic toolbox for the production of pharmaceutically-relevant triterpenes**
Christelle ANDRÉ – (MC member LU) Luxembourg Institute of Science and Technology, Belvaux (LU)

18.15 OC19 **New efficient inhibitors of aldose reductase from the family of carboxymethylated indoles**
Magdaléna MÁJEKOVÁ – (MC member SK) Center of Experimental Medicine, Bratislava (Slovakia)

18.30 OC20 **Macrocyclic amidinoureas, a valid scaffold for antifungal and chitinase inhibiting compounds**
Francesco OROFINO – Università degli Studi di Siena (Italy)

20.30 *Social Dinner at TASCA SIN FRENO, located at Calle Cruz de la Candelaria 35, La Laguna*

## Friday March 16th 2018

**Session V "Development of Computational methods for multiple ligand design and discovery within WG4"**
**Moderator: Hanoch Senderowitz (MC member Israel, WG4 leader)**

9.00 PL4 **MD Derived Feature-based Pharmacophores: A New Way to Enhance Virtual Screening Efficacy in Multitarget Drug Discovery**
Thierry LANGER (MC substitute A) - University of Vienna (Austria)

9.30 OC21 **Efficient virtual screening and prediction of binding profiles**
Olli PENTIKÄINEN (new MC member FI) - University of Turku (Finland)

9.45 OC22 **Novel techniques for multitarget and multi-antitarget design for blebbistatin analogs**
Alfonso GARCIA-SOSA (MC member EE, comunication manager CA15135) - University of Tartu (EE)

10.00 OC23 **Self-assembled ligands targeting TLR7: a molecular level investigation**
Andrea DANANI – (MC substitute CH) Università della Svizzera Italiana (USI), Manno (Switzerland)

10.15 OC24 **Induced Fit Docking Protocol applied to the *in silico* evaluation of antiviral HIV-1 integrase resistance**
Francesca Alessandra AMBROSIO - Università "Magna Græcia" di Catanzaro (Italy)

10.30 OC25 **STD-NMR-in silico Screening: An Effective Combination for Validating ChE and MAO-B Dual Inhibition**
Antony BURKE – Universidade de Évora (Portugal)

10.45 OC26 **Multi-level strategy for analysis of bioactive drug conformations**
Sanja ZIVANOVIC –The Barcelona Institute of Science and Technology, Barcelona (ES)

11.00 *Coffee break*

11.30 **Round table and best poster awarding ceremony**
**Moderator: Fernanda BORGES (CA15135 Vice-Chair) - University of Porto (Portugal)**
Danijel KIKELJ (WG1 leader) - University of Ljubljana (Slovenia)
Eugenio GAUDIO (WG2 leader) - Oncology Research Institute, Bellinzona (Switzerland)
Sharon BRYANT (WG3 leader) - Inte:Ligand GmbH, Vienna (Austria)
Hanoch SENDEROWITZ (WG4 leader) - Bar-Ilan University, Ramat-Gan (Israel)
Alfonso GARCIA-SOSA (MC member EE, comunication manager CA15135) - University of Tartu (EE)
Maria Laura BOLOGNESI (STSM coordinator CA15135) - "Alma Mater" Università di Bologna (Italy)
Simona RAPPOSELLI (Associate Editor Frontiers in Chemistry) - Università di Pisa (Italy)
Claire SHOEMAKE (Gender and Inclusiveness coordinator) - University of Malta (Malta)

12.30 **Concluding remarks**

13.00 **Core meeting (for the MuTaLig Core group only)**

# Plenary lectures

## Plenary Lecture 1

# New molecular entities as inhibitors of HBV and (re)emerging virus replication

María- José Camarasa,[a]

[a] *Instituto de Química Médica (IQM, CSIC), Juan de la Cierva 3, 28006 Madrid (Spain)*

*mj.camarasa@iqm.csic.es*

Infectious diseases have been a cause of concern for humans over millennia and nowadays the expansion of an outbreak in one part of the world is often unpredictable as it is facilitated by the ease of world travel and the global exchange of goods. Two major categories of infections can be defined, namely, newly emerging diseases that are recognized in the human host for the first time and reemerging diseases that historically have infected humans but continue to appear in new locations or in drug-resistant forms, or reappear after apparent control or elimination, often accompanied by significant changes in pathogenicity (HIV infection is one example of emerging viral infections). These diseases have a profound global economic and social impact in relation to illness-related deaths and also in the interference with normal life activities. Due to the capacity of RNA viruses for rapid mutation these viruses are particularly prone to adaptation to environmental changes. In addition, the mutability of RNA viruses is one of the reasons that explain the lack of efficacy of vaccination against these pathogens.

For a number of years our research group has been deeply involved in the design and discovery of new antivirals, an area in which there are still largely unmet medical needs affecting humans, particularly highly prevalent pathologies such as AIDS, and more recently in HVB and (re)emerging pathogens that seriously compromise human health. In this presentation a medicinal chemistry approach followed by our group to rationally design inhibitors of the replication of two different (re)emerging RNA virus, namely, chikungunya and enterovirus 71 and two highly prevalent emerging viruses as HIV and HBV will be discussed.

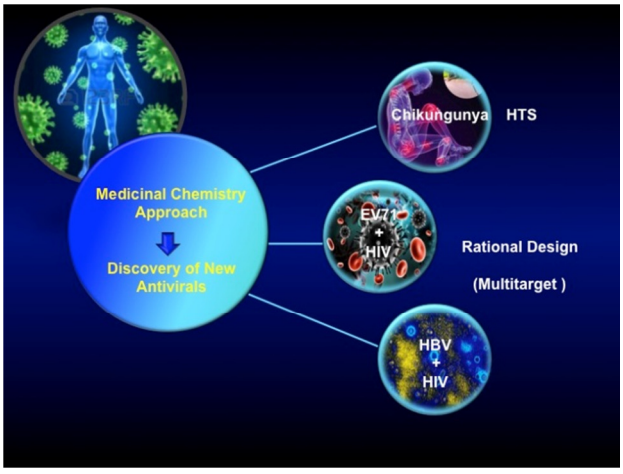


**Figure 1:** Discovery of new antivirals

Acknowledgments

The Spanish MICINN/MINECO SAF2012-39760-C02; SAF2015-64629-C2-1-R (MINECO/FEDER) and the Comunidad de Madrid (BIPEDD-2-CM ref. S-2010/BMD-2457) are acknowledged for financial support.

## Plenary Lecture 2

# MicroRNAs in Oncogenesis: Leadings or Passengers?

# It doesn't matter!

Rami Aqeilan

*Dept of Immunology & Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel ramiaq@mail.huji.ac.il*

Protein-coding genes comprise only 3% of the human genome, while the fast majority of the genome is comprised of non-coding genes; RNAs that do not code for proteins. MicroRNAs (miRNAs) are short non-coding RNAs that play critical roles in numerous cellular processes through post-transcriptional regulating functions. During the last decade, we and others have reported that unique miRNA signatures associate with the pathogenesis and progression of several types of cancer. MiRNAs can act as tumor suppressors or behave as oncogenes depending on cellular context. In the last few years, our attempts were focused to design potent miRNAs as anticancer drugs and drug targets. Typically, one strand of a miRNA duplex is bound by argonaute proteins, loaded on miRNA-induced silencing complex (miRISC), and guides the miRISC to target mRNAs. This strand is called "lead" or "guide" strand. The other strand is usually mostly degraded and presented in the cell at much lower level. This strand is called "passenger" or "star" strand and designated as miR*. We recently found that the passenger strand of miRNAs (miR*) can have potent biological effects. We demonstrated that, for example, miR-16-1* and miR-16-2* inhibits primary tumor growth, metastasis, and chemoresistance and invasiveness of human cancer cells. Noteworthy, star miRNAs have different, although strongly overlapping functions with leading strand miRNAs. Importantly, *in vivo* systemic delivery of miR* on its own or in combination with chemotherapy has promising anti-tumor effects which prompt us to expand use of miR* in clinical trials for the treatment of relevant cancer types. Our findings indicate that deregulation of miRNA expression is a driving force in oncogenesis that can be utilized to target tumor cells.

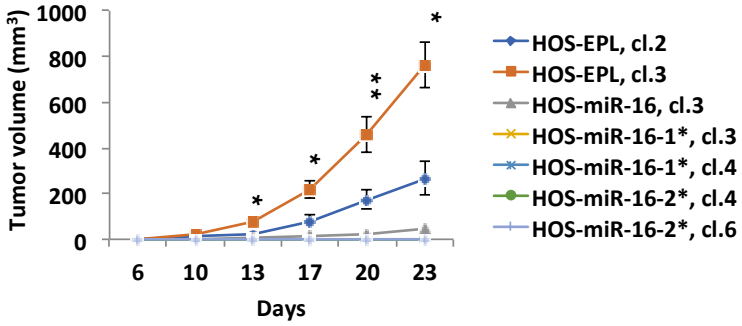


**Figure 1: Effects of human miR-16, miR-16-1* and miR-16-2* overexpression on human osteosarcoma cells tumorigenesis *in vivo*.** HOS osteosarcoma cells overexpressing corresponding miRNAs were subcutaneously injected in flank of NOD/SCID mice. HOS-EPL, cl. 2 and HOS-EPL, cl. 3, which are infected with the empty lentivirus, were applied as controls. (A) Time course of tumor growth for all HOS clones is presented.

References

Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differ. 2010 Feb;17(2):215-20.

Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo GJ, Lovat F, LeBlanc K, Palatini J, Randall RL, Volinia S, Stein GS, Croce CM, Lian JB, Aqeilan RI. miRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer Res. 2012 Apr 1;72(7):1865-77.

Salah Z, Arafeh R, Maximov V, Galasso M, Khawaled S, Abou-Sharieha S, Volinia S, Jones KB, Croce CM, Aqeilan RI. miR-27a and miR-27a* contribute to metastatic properties of osteosarcoma cells. Oncotarget. 2015 Mar 10;6(7):4920-35.

## Plenary Lecture 3

# Computational Studies of the Binding Selectivity of a Potent Human Asparagine Synthetase Inhibitor

Nigel G. J. Richards,[a] and Ashish Radadiya.[a]

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Cancer cells require the adequate provision of energy and nutrients to support cell growth, consistent with the idea that alteration of key metabolic processes often enhance tumorigenesis. As a result, "nutrient deprivation" has become a promising strategy for anti-cancer therapies. Several very recent studies have shown that L-asparagine is a key metabolic nutrient for solid tumors including sarcoma, breast cancer, hepatocellular carcinoma and castration-resistant prostate cancers. Validating ASNS as a cancer target requires, however, access to potent cell-permeable, highly selective, small molecule ASNS inhibitors. This lecture will discuss the use of a high-resolution X-ray crystal structure of human ASNS in computational studies aimed at delineating the mode of interaction between a sulfoximine-based inhibitor and the enzyme. In addition, the results of an activity-based assay will be presented that establish that this inhibitor is highly selective in its interactions with the cellular proteome. The final part of the lecture will outline additional computational studies aimed at evaluating whether the ASNS inhibitor will interact with the enzyme $NAD^+$ synthetase, which is also a drug target.

References

[1] Ikeuchi, H.; Ahn, Y.; Otokawa, T.; Watanabe, B.; Hegazy, L.S.; Hiratake, J.; Richards, N.G.J. A human asparagine synthetase inhibitor kills asparaginase-resistant MOLT-4 cells. *Bioorg. Med. Chem.* **2012**, *20*, 5915-5927.

[2] Hettmer, S.; Schinzel, A.; Tssechalova, D.; Bronson R.T.; Richards, N.G.J.; Hahn, W. C.; Wagers, A.J. Functional genomic screening reveals asparagine dependence as a metabolic vulnerability in sarcoma. (2015) *eLife* **2015**, 10.7554/eLife.09436.

[3] Rosenblum, J.S.; Nomanbhoy, T.K.; Kozarich, J.W. Functional interrogation of kinases and other nucleotide-binding proteins. *FEBS Lett.* **2013**, *587*, 1870-1877.

## Plenary Lecture 4

# MD Derived Feature-based Pharmacophores: A New Way to Enhance Virtual Screening Efficacy in Multitarget Drug Discovery

Thierry Langer

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Pharmacophore-based compound modeling, virtual screening, and bio-activity profiling has become one of the most popular in silico techniques for supporting medicinal chemists in their hit finding, hit expansion, hit to lead, and lead optimization programs. The molecular design tool LigandScout[1] has been developed to address successfully one of the most important issues in virtual screening: Enhancing early enrichment while maintaining high computational speed as well as ease of use, as shown by reference studies.[2] As an extension of the static pharmacophore approach, we lately have focused on incorporating dynamic effects of ligand protein binding into our automated interaction determination process.

The Common Hits Approach (CHA)[3] developed in our group uses the multiple coordinate sets saved during MD simulations and generates for each frame a pharmacophore model. Pharmacophore models with the same pharmacophore features are pooled, thus reducing the high number of initially obtained models to only a few hundred *representative* pharmacophore models. Virtual screening runs are then performed with every representative pharmacophore model and the screening results are combined and re-scored to generate a single hit list. The score for a particular molecule is finally calculated based on the number of representative pharmacophore models, which classified a particular molecule as being active.

On a validation data set of a representative selection of protein ligand complexes we demonstrate that early enrichment and AUC obtained by this approach outperforms classical structure-derived pharmacophore based virtual screening.

References

[1] Wolber G, Langer T, *J Chem Inf Model.* **2005**; 45(1): 160-69.

[2] Karaboga AS, Planesas JM, Petronin F, Teixido J, Souchet M, Perez-Nueno VI. *J Chem Inf Model.* **2013**; 53(3):1043-56.

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# Short communications

## Short communication 1

# Novel indanone derivatives as MAO B/$H_3$R dual targeting ligands for treatment of Parkinson's disease

Stark, Holger,[a] Anna Affini,[a] Aleksandra Zivkovic,[a] Jose Marco-Contelles,[b] Stefanie Hagenow[a]

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The design of multi targeting ligands was developed in the last decades as an innovative therapeutic concept for Parkinson's disease (PD) and other neurodegenerative disorders. As the monoamine oxidase B (MAO B) and the histamine $H_3$ receptor ($H_3$R) are promising targets for dopaminergic regulation,[1] we synthetized dual targeting ligands (DTLs) as non-dopaminergic receptor approach for the treatment of PD.[2] Three series of compounds were developed by attaching the $H_3$R pharmacophore to indanone-related MAO B motifs,[3] leading to development of MAO B/$H_3$R DTLs.

Among synthesized indanone DTLs, compounds bearing the 2-benzylidene-1-indanone core structure showed MAO B preferring inhibition capabilities along with nanomolar h$H_3$R affinity. Substitution of C5 and C6 position of the 2-benzylidene-1-indanones with lipophilic substituents revealed three promising candidates exhibiting inhibitory potencies for MAO B with $IC_{50}$ values ranging from 1931 nM to 276 nM and high affinities at h$H_3$R ($K_i$ < 50 nM). Compound **3f** (MAO B $IC_{50}$ = 276 nM, h$H_3$R $K_i$ = 10 nM) showed highest preference for MAO B over MAO A (SI > 36). Interestingly, $IC_{50}$ determinations after preincubation of enzyme and DTLs revealed also nanomolar MAO B potency for **3f**s structural isomer **3e** (MAO B $IC_{50}$ = 232 nM) and **3d** (MAO B $IC_{50}$ = 541 nM), suggesting time-dependent inhibition modes. Reversibility of inhibition for all three compounds were confirmed by dilution studies in excess of substrate. Thus, indanone-substituted derivatives are promising lead structures for the design of MAO B/h$H_3$R DTLs as novel therapeutic approach of PD therapy.

References

[1] 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*, 1–17.

[2] Bautista-Aguilera, O. M.; Hagenow, S.; Palomino-Antolin, A.; Farré-Alins, V.; Ismaili, L.; Joffrin, P.-L.; Jimeno, M. L.; Soukup,O.; Janočková, J.; Kalinowsky, L.; Proschak, E.; Iriepa, I.; Moraleda, I.; Schwed, J. S.; Romero Martínez, A.; López-Muñoz, F.; Chioua, M.; Egea, J.; Ramsay, R. R.; Marco-Contelles, J.; Stark, H. Multitarget-Directed Ligands Combining Cholinesterase and Monoamine Oxidase Inhibition with Histamine H3R Antagonism for Neurodegenerative Diseases, *Angew. Chemie Int. Ed.* **2017**, *56*, 12765-12769.

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## Short communication 2

# Biological activity of novel benzylidene-based (1,3-selenazol-2-yl)hydrazones

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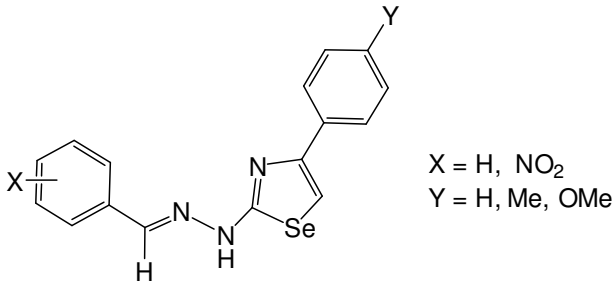
Biological studies were performed on a focused library of 12 novel benzylidene-based (1,3-selenazol-2-yl)hydrazones (Scheme 1). The compounds showed excellent free radical scavenging activities and oxygen radical absorption capacities in DPPH and ORAC assays. Some of the compounds showed better antiproliferative activity than a blockbuster chemotherapeutic 5-fluorouracil. Some of the novel benzylidene-based (1,3-selenazol-2-yl)hydrazones are structural analogues of previously reported human MAO-B inhibitors from the class of (1,3-thiazol-2-yl)hydrazones.[1] Therefore, activity of the investigated compounds against both MAO-A and MAO-B was additionally determined.




**Scheme 1:** Benzylidene-based (1,3-selenazol-2-yl)hydrazones studied in this work.

Acknowledgements

N.R.F. and S. H. thank MuTaLig COST Action CA15135 for an STSM.

References

[1]Secci, D; Bolasco, A; Carradori, S; D'Ascenzio, M; Nescatelli, R; Yáñez, M. Recent advances in the development of selective human MAO-B inhibitors: (Hetero)arylidene-(4-substituted-thiazol-2-yl)hydrazines. *Eur. J. Med. Chem.,* **2012**, *58*, 405-4171.

## Short communication 3

# The utility of the xanthine scaffold for developing MAO-B inhibitors and antioxidants

David Synak,[a] Agata Doroz-Płonka,[a] Agnieszka Olejarz,[a] Gniewomir Latacz,[a] Jakub Schabikowski,[a] Christa E. Müller,[b] Katarzyna Kieć-Kononowicz[a]

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Two hundred years after the first report on Parkinson's Disease (PD) this and other neurological disorders still pose a significant challenge in modern medicine.[1] While the pathogenesis and the brain areas affected by these diseases differ, a common theme seems to be an elevated level of oxidative stress.[2] In the case of PD, at least part of the cause for this redox imbalance seems to be the catalytic activity of monoamine oxidase B (MAO-B), which generates $H_2O_2$ as a side product of the oxidative deamination of dopamine. Since dopamine levels are increased when treated with levodopa, this leads to an amplification of the redox imbalance. Therefore, development of compounds which both inhibit MAO-B and also act as antioxidants should prove useful in future treatment of the disease combined with levodopa.

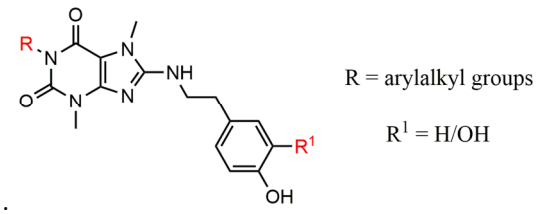


**Figure 1:** General structure of the evaluated compounds

Based on literature[3] and earlier research, a series of novel compounds derived from the xanthine scaffold has been designed, synthesized and evaluated for MAO-B inhibition and antioxidant properties. These structures consist of the theobromine scaffold substituted at the 8-position by dopamine or *p*-tyramine and at the N1-position by different arylalkyl substituents (Figure 1). The synthesis involved a 4- to 5-step procedure, yielding the final compounds with high purity. Their identities were confirmed by spectroscopic analyses. The compounds' potential to inhibit MAO-B was determined using human recombinant MAO-B and the Amplex Red monoamine oxidase kit, while the antioxidant properties were evaluated via the FRAP (Ferric reducing ability of plasma) test, with results being compared to ascorbic acid as a reference.

Details regarding design, synthesis and biological assays will be presented.

Financial support by the Jagiellonian University Medical College grant no. K/ZDS/007121, and the MuTaLig COST Action (CA15135) are gratefully acknowledged.

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## Short communication 4

# Chalcogen-based privileged structures in the design of new multitarget drugs

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The high complexity exhibited by cancer and neurodegenerative diseases has forced the development of multitarget drugs for tackling their multifactorial nature. In this context, we have reported that the combination of chalcogen-containing motifs with a panel of pharmacophores (e.g. polyphenols, iminosugars, steroids, tacrine)[1–5] can provide lead compounds with relevant biological properties. Herein we present our recent results in the preparation of tacrine-based homo- and heterodimers that incorporate an antioxidant tether (selenoureido, chalcogenide) as promissing dual compounds for the treatment of Alzheimer's disease (inhibitors of AChE, inhibitors of amyloid-β self aggregation, low neurotoxicity) and as antiproliferative agents (Figure 1).


**Figure 1.** General structure of the multipotent chalcogen-containing pharmacophores against degenerative diseases

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## Short communication 5

# Development of multi-target agents based on hydroxybenzoic acid scaffold endowed with mitochondriotropic antioxidant and cholinesterase inhibitory activities

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

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

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

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

## Short communication 6

# Naphthalimide Molecular Logic Gates as Fluorescence Cellular Imaging and Anticancer Agents

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The development of naphthalimide (benz[*de*]isoquinolin-1,3-diones)[1] and ferrocene-based[2] compounds are currently perceived as independent topics in cancer research. Our group has taken the initiative to develop novel derivatives based on naphthalimide and ferrocene as synergistic functional optical materials[3] and as potential therapeutic cancer agents.[4] Working towards this goal, we have designed a series of naphthalimide-based molecules incorporating a tertiary amine, which is susceptible to protonation for improved water solubility, and a ferrocenyl moiety for promoting redox chemistry.[5] In this presentation, we will highlight the synthesis, cytotoxicity and fluorescent cell imaging in MCF-7 and K562 cells for this new class of compound as represented in Figure 1.

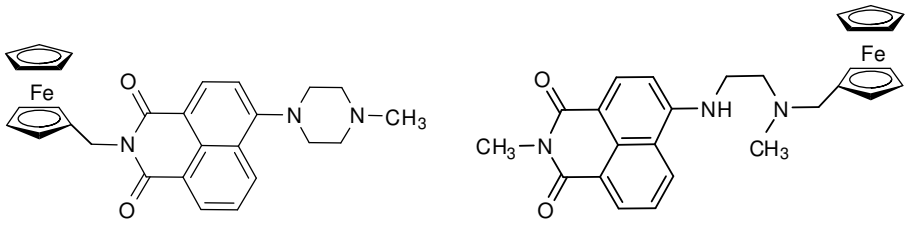


**Figure 1:** Examples of synergistic cytotoxic and fluorescence imaging agents.

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## Short communication 7

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

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This research takes place in the field of cancer drug development. The fundamental problem is that antitumor efficacy, in preclinical cancer models, does not translate faithfully to patient outcomes. Cancer drug discovery is generally performed under in vitro conditions in cell-based models that poorly represent actual malignancies. On our platform we currently run 3D cultures of different human cell line models (testis CSC, pancreatic CSC, blood and liver carcinoma, plus fibroblast as control cells), a new anti-cancer therapeutic approach to study in detail the differential activities. Testing in checkerboard different sets of 3 drugs will allow us to determine the best ways to induce apoptosis with the lowest drug concentrations. In the current project, we will study several sets of new bioactive compounds in combination or not with traditional market drugs. The new active compounds we identified earlier demonstrate the potential for finding new lead compounds for drug research. Hybrid drug molecules will be designed to counterbalance side effects associated with the other hybrid part or to amplify its effect through action on another bio target or to interact with multiple targets as one single molecule lowering the risk of drug-drug interactions and minimizing the drug resistance. In conclusion, it is safe to say that the risk of failure of this project is minimized due to the choice of in vitro three-dimensional (3D) culture systems of tumor cells, a new area of interest in cancer research, since their complexity and pathophysiology resembles more the in vivo tumor tissue in their cell responses to resist to chemotherapies.

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## Short communication 8

# Symmetry of Ligands: Matriptase-2 Inhibitors as a Case Study in Drug Design

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For structure-based drug design, specific features of binding sites in different targets play a major role in rationalizing ligand binding characteristics. For example, dibasic compounds have been reported as potent inhibitors of various trypsin-like serine proteases, the active sites of which contain several binding pockets that can be targeted by cationic moieties. This results in several possible orientations within the active site, complicating the binding mode prediction of such compounds by docking tools. We introduced symmetry in bi- and tribasic compounds to reduce conformational space in docking calculations and to simplify binding mode selection by limiting the number of possible pocket occupations. Asymmetric bisbenzamidines were used as starting points for a multistage and structure-guided optimization. A series of 24 final compounds with either two or three benzamidine substructures was ultimately synthesized and evaluated as inhibitors of five serine proteases, including matriptase-2. This study underlines the relevance of ligand symmetry for chemical biology.[1]

Matriptase-2, a type II transmembrane serine protease, plays a key role in the human iron homeostasis and is considered as an attractive target for the treatment of iron overload diseases, such as hemochromatosis and β-thalassemia. Certain peptide phosphonates have been developed and kinetically characterized irreversible inhibitors of matriptase-2. In addition to a phosphonate warhead, these dipeptides possess two benzguanidine moieties as arginine mimetics to provide affinity for matriptase-2 by binding to the S1 and S3/S4 subpockets, respectively. One derivative with (*S*)-configuration at the N-terminal amino acid and (*R*)-configuration at the phosphonate carbon was the most potent matriptase-2 inactivator with a $k_{inac}/K_i$ value of 2790 $M^{-1}s^{-1}$ and abolished the activity of membrane-bound matriptase-2 on the surface of intact cells. Based on the chemotype of phosphono *bis*-benzguanidines, the design and synthesis of the first activity-based probe for matriptase-2 was carried out and the *in-gel* fluorescence detection of matriptase-2 was demonstrated.[2]

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## Short communication 9

# The propargylamine warhead in multi-target compounds for inactivation of MAO

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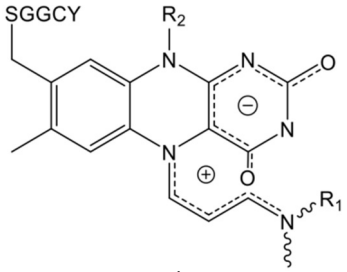
Incorporation of multiple functionalities into a new molecular entity to give a multi-target-directed ligands (MTDLs) requires careful biological assessment to ensure that the specific target effects are not significantly altered and that the kinetic behaviour remains favourable within the MTDL. Combinations of inhibitors of monoamine oxidases (MAO) and the cholinesterases (ChE) have potential uses in neurodegenerative disease, but the need to balance partial inhibition of ChE with at least 80% inhibition of MAO is challenging. The combination of reversible inhibition of ChEs with irreversible inhibition of MAOs that has proven useful in rasagiline is a common strategy. We have characterised the kinetics of inactivation of several standard propargylamine inactivators of MAOs to guide the design of MTDL combinations not only with ChE inhibition but also with activity at receptors.

**Figure 1:** Structure of the ASS234-Flavin pentapeptide adduct after digestion of MAO

In addition to kinetic studies, the adduct of MAO-A with a propargylamine has been analysed using a combination of ultra-high performance liquid chromatography, spectroscopy, and mass spectrometry. The covalent cyanine structure




linking the multi-target propargylamine inhibitor ASS234[1] and the flavin adenine dinucleotide in MAO, has a partial double bond character that gives rise to 4 interconverting geometric isomers. The findings provide key information relevant for the use of the propargyl moiety in novel multi-target drugs, and support a new mechanism of MAO inactivation applicable to all propargylamine inhibitors.

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## Short communication 10

# Hydrogen sulfide: a candidate multitarget ligand for cardiovascular diseases.

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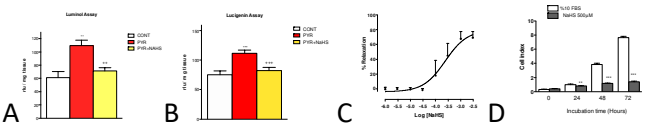
Cardiovascular diseases such as hypertension, atherosclerosis and cardiac failure are among the leading causes of death in the world. Vascular contraction, oxidative stress, vascular smooth muscle proliferation and inflammation are the main problems in cardiovascular diseases. Thus targeting these processes with one drug will be a good option for treatment of atherosclerosis and vascular diseases. Hydrogen sulfide ($H_2S$) is recently recognized as a neurotransmitter and the level of $H_2S$ has been found to decrease in cardiovascular diseases (1). $H_2S$-donating drugs are now testing in phase 2 studies as anti-inflammatory drug (2). We hypothesized that $H_2S$ donor sodium hydrogen sulfide (NaHS) can inhibit oxidative stress, vascular contraction and vascular smooth muscle proliferation and therefore $H_2S$ donors or even $H_2S$-donating cardiovascular drugs may be good candidates as multitarget ligands for cardiovascular diseases. We tested the effect of NaHS on vascular relaxation and reactive oxygen species (ROS) of mice aorta, proliferative effects of fetal bovine serum in primary rat thoracic aortic vascular smooth muscle cells. One way or Two way Anova was used as statistical test, where it is appropriate. Mice aortic rings were incubated with vehicle or NaHS (1 mM, 10 min.) as treatment, pyrogallol (0.1 mM, 5 min) was used to induce oxidative stress and then ROS were measured by luminol-lucigenin assay (3). The vasorelaxant effect of NaHS was evaluated in endothelium intact, phenylephrine pre- contracted (10 µM) mice aortic rings by DMT myograph. NaHS significantly inhibited vascular oxidative stress ($p<0.05$, n=7) and phenylephrine-induced contraction, dose dependently. Real time cellular analyzes by xCELLigence showed that NaHS (0.5 mM, 24-72 h) inhibits 10% FBS-induced proliferation of rat thoracic aorta cells significantly ($p<0.05$, n=3-4). These results shows that $H_2S$ releasing drugs may present a better candidate for the treatment of atherosclerosis, ischemia or hypertension, since they have a capacity to inhibit smooth muscle proliferation, oxidative stress and vascular contraction. Our study may be important to show the importance of $H_2S$ to synthesize multitarget ligands for cardiovascular diseases such as hybrid molecules of $H_2S$-donating antihypertensive drugs or hypo-cholesterolemic drugs. Besides $H_2S$-donating multifunctional drugs may have an advantage to avoid being a high-molecular weight molecules exhibiting poor "drug-likeness" (4). **Acknowledgement**: We thank Turkish Scientific Research Council TUBITAK for the grant #114S448, COST action CA15135, 14ECZ05 and 14ECZ026.

**Figure 1:** The effect of $H_2S$ donor, NaHS on A) Reactive oxygen species B) Superoxide radicals induced by pyrogallol (0.1 mM, 5 min.) C) Vascular contraction induced by phenylephrine in mice aorta and D) Proliferation induced by %10 FBS in aortic smooth muscle cell.

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## Short communication 11

# Combinatorial screening of the pan-PI3K inhibitor Copanlisib in T cell lymphomas

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Introduction. T cell lymphomas represent a group of disorders for which therapeutic improvements are still widely needed. Here, we performed a screening of copanlisib, a pan class I phosphatidylinositol-3-kinase (PI3K) inhibitor (i) with predominant inhibitory activity against both PI3K-δ and PI3K-α isoforms, as single agent and in combination with several other anti-cancer agents on a panel of cell lines derived from anaplastic large cell lymphoma (ALCL), peripheral T cell lymphoma (PTCL) and Sèzary syndrome (SS).

Methods. Cell lines derived from ALCL (SU-DHL-1, L82, MAC1, KARPAS299, KI-JK), PTCL (FEPD, HH) and SS (H9, HUT78) were exposed to increasing doses of copanlisib alone and in combination with increasing doses of other compounds using the fixed ratio set-up. Tested compounds were: anti CD30 antibody-drug conjugate brentuximab, ALK-i crizotinib, CDK-i roniciclib, DNA damage agent bendamustine, HDAC-i panobinostat and romidepsin, immunomodulatory lenalidomide, JAK1/2-i ruxolitinib, BTK-i ibrutinib, MALT-i MI2, proteasome-i bortezomib, BCL2-i venetoclax, CDK4/6-i palbociclib, the BET-i BAY 1238097, the PTEFb/CDK9-i BAY 1143572. Synergy was assessed with Chou-Talalay combination index (CI): synergism (<0.9), additive (0.9-1.1), antagonism/no benefit (> 1.1). Gene expression profiling was done using the Illumina-HumanHT-12 Expression-BeadChips and GSEA (FDR<0.25).

Results. Copanlisib had a median IC50 of 285 nM (50-1660 nM). Among the other compounds, the most active were bortezomib (IC50 3.1 nM; 1.6-6 nM), romidepsin (IC50 2.4 nM; 1.8-7.7 nM), panobinostat (IC50 10.2 nM, 3.8-14 nM), roniciclib (IC50 21.1 nM, 13.4-50.1 nM). Different copanlisib-containing combinations (Table 1), tested in the 9 cell lines, were synergistic: copanlisib with palbociclib (7/9 cell lines), panobinostat (7/9), BAY 1238097 (6/9), venetoclax (5/9), romidepsin (5/12), ruxolitinib (4/12), lenalidomide or BAY 1143572 or brentuximab or crizotinib (3/9). The most promising combinations were copanlisib/venetoclax and copanlisib/palbociclib, with a median CI<0.7 in 3 cell lines. High expression of genes involved in interferon signaling and TP53 pathway were associated with synergism to copanlisib/venetoclax, while MYC target genes and cell cycle signaling were associated with resistance to the combination. Largely the opposite was observed for copanlisib/palbociclib, with synergism in cells with high expression of E2F targets and genes involved in cell cycle and resistant in cells with expression of transcripts involved in interferon and TP53 signaling.

| Combination with Copanlisib | Percentage of cell lines in which combination were beneficial* | 95% Conf. Interval | Mechanism of action of combination partner |
|---|---|---|---|
| Panobinostat | 89% (8/9) | 0.52 – 0.99 | HDAC inhibition |
| BAY 1238097 | 89% (8/9) | 0.52 – 0.99 | BET inhibition |
| Palbociclib | 78% (7/9) | 0.40 – 0.97 | CDK4/6 inhibition |
| Venetoclax | 67% (6/9) | 0.30 – 0.92 | BCL2 inhibition |
| Romidepsin | 67% (6/9) | 0.30 – 0.92 | HDAC inhibition |
| Crizotinib | 67% (6/9) | 0.30 – 0.92 | ALK and MET inhibition |
| Lenalidomide | 56% (5/9) | 0.21 – 0.86 | immunomdulation |
| BAY 1125976 | 44% (4/9) | 0.14 – 0.79 | AKT1/2 inhibition |
| Roniciclib | 44% (4/9) | 0.14 – 0.79 | CDK inhibition |
| Bendamustine | 44% (4/9) | 0.14 – 0.79 | chemotherapy |
| Ruxolitinib | 44% (4/9) | 0.14 – 0.79 | JAK1/2 inhibition |
| Brentuximab | 44% (4/9) | 0.14 – 0.79 | Anti CD30 - ADC |
| MI2 | 33% (3/9) | 0.07 – 0.7 | MALT1 inhibition |
| BAY 1143572 | 33% (3/9) | 0.07 – 0.7 | PTEFb/CDK9 inhibition |

**Figure 1:** Copanlisib–containing combinations in 9 cell lines derived from ALCL, PTCL, SS. *, synergistic or additive according to the Chou-Talalay algorithm.

Conclusion. Copanlisib was active in T-cell lines derived from ALCL, PTCL and SS. The combinations with the BCL2-inhibitor venetoclax and with the CDK4/CDK6-inhibitor were the most synergistic and the specific GEP features might predict lymphomas that could benefit from these regimens.

## Short communication 12

# Metformin interference with survival signaling in DLBCL

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Metformin, the most prescribed oral anti-diabetic drug in the world, is now extensively evaluated as a repurpused anti-cancer agent. Numerous studies have shown that metformin decreases cancer risk in diabetic patients (1,2) that have led to a large number of clinical trials utilizing anticancer effects of metformin as mono-therapy or in combination with different agents in cancer patients (3). To evaluate capability of metformin to multi-target key molecules in survival pathways (including NFkB, Mek-ERK and PI3K/Akt/mTOR signaling), we treated diffuse-large B cell lymphoma (DLBCL) cell lines with metformin alone or in combination with selected inhibitors and novel anti-lymphoma agents. For selected inhibitory compounds that are currently in clinical trials, including four anti-lymphoma drugs (BTK inhibitor ibrutinib, the HDAC inhibitor vorinostat, the PI3K delta inhibitor idelalisib, the Bromodomain BRD2/3/4 inhibitor OTX015) we have determined IC50 concentrations in four different lymhoma cell lines.

Western blotting was used for evaluating the activity of target kinases in survival signaling cascades after the treatment and co-treatment. We found that metformin exhibited activity in all tested DLBCL cell lines and have shown additive or synergic inhibitory effects even at IC10 combinations. Metformin alone or in co-treatment successfully inhibited the activity of Akt and NFkB signaling together with their downstream substrates.

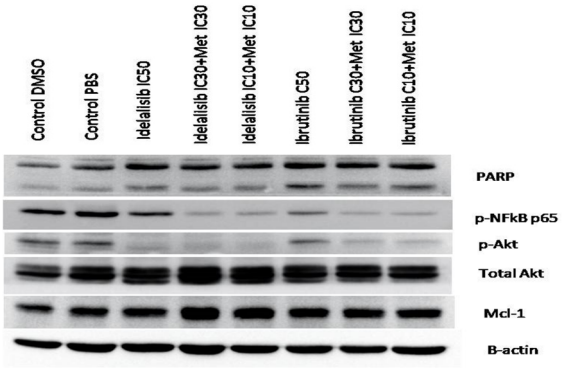


**Figure 1.** Effects of metformin in HBL cell line

*Results were obtained thanks to successful STSM, supported by MuTaLig COST Action CA15135.*

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## Short communication 13

# Mitochondriotropic antioxidants based on cinnamic acid increase cellular stress responses in HepG2 cells

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**Background:** There is accumulating evidence that mitochondrial dysfunction, including disruption of the respiratory chain and excessive production of reactive oxygen species (ROS), plays a key role in the physiopathology of hepatic diseases. Mitochondrial dysfunction can also lead to apoptosis or necrosis depending on the energy status of the cell, resulting in inflammation. Mitochondria-targeted therapies based on bioactive molecules bound to, namely involving lipophilic cations carriers that can cross mitochondrial membranes and accumulate within the mitochondrial matrix are being developed. In this context, we have produced novel mitochondrial-directed antioxidant based on naturally occurring phenolic acids. Our current hypothesis is that non-toxic concentrations of these novel molecules activate cellular intrinsic antioxidant defenses, similarly to the ability of parent phenolic acids activate survival and antioxidant cell responses.

**Material and Methods:** We studied the effects of the novel mitochondriotropic agents ($AntiOxCIN_4$ and $AntiOxCIN_6$) developed by our group on human hepatoma-derived cell line HepG2, namely regarding cell viability, cell mass, ROS levels, caspase 3- and 9-like activities, glutathione (GSH) content, peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1-α) and superoxide dismutase (SOD) expression.

**Results:** We performed an initial cytotoxicity screening to identify non-toxic concentrations of the novel molecules. Our data showed that positively charged cations with catechol moieties presented higher toxicity than those containing a pyrogallol moiety in their structure. After 48h of treatment, $AntiOxCIN_4$ caused an increase of 20% on HepG2 cell mass and induced a transient increase in intracellular ROS without triggering pro-apoptotic responses. $AntiOxCIN_4$ also prompted a ROS-dependent stimulation of the endogenous antioxidant defense system and mitochondrial biogenesis, as measured by an increase in GSH content and PGC1-α expression, respectively.

**Conclusions:** Mitochondriotropic antioxidants based on dietary scaffolds with hormetic-like effects, displaying indirect antioxidant mechanisms, can be used as therapeutic agents in the treatment of oxidative stress-related conditions.

**Acknowledgements:** This work was funded by FEDER funds through the Operational Programme Competitiveness Factors - COMPETE and national funds by FCT - Foundation for Science and Technology under research grants PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440. J. Teixeira (NORTE-01-0145-FEDER-000028), R. Amorim (PTDC/DTP-FTO/2433/2014), F. Cagide (SFRH/BPD/74491/2010) and S. Benfeito (SFRH/BD/99189/2013) grants are supported by the European Regional Development Fund (ERDF) through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT–Fundação para a Ciência e a Tecnologia.

## Short communication 14

# Unbiased assessment of direct or indirect targets of any bioactive compound on a proteome-wide scale

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The identification of drug targets is still one of the biggest challenges of drug discovery today. The rationale design or discovery of multi-targeted drug require to assess all direct or indirect target proteins of the compound on a proteome-wide scale. A mass spectrometry based method has been recently developed that study the perturbation of the thermal stability of the proteome[1]. It has been successfully applied to study drug targets and off-targets [2-4]. It is based on the principle that a protein complexed to a ligand become more resistant against heat-induced unfolding. Any bioactive compound that can alter the melting point of a protein can be considered a binder of that protein. This approach is called Thermal proteome profiling[1].

Thermal proteome profiling is a very promising strategy to prediction of direct and indirect targets of any drug or bioactive compound because thousands of candidate protein targets can be assessed in parallel. Another advantage is the ability to assess the compound-cellular protein interactions in a physiologically relevant context. It can be analyzed in any cell extract, intact cells or tissues. The detection method is based on quantitative mass spectrometry and therefore providing data from the entire cellular proteome without additional requirements of any specific antibodies. This approach also eliminate the problem from classical phenotypic screenings because the identified direct and indirect targets could be map the entire targeted pathways of the studied compound. This knowledge is the first step to elucidate possible mode of action of a compound or drug and the interactions of the different protein targets in a living cells that is the realistic scenario. Moreover, additional experiments can be performed to determine compound potency and to infer a rank of potency of the different protein targets at the cellular context.

Our lab is involved in two projects where this methodology is applied: i) Cyanobesity, from ERA-NET. Marine biotechnology. In this project novel bioactive compound from cyanobacterial has been selected in phenotypic screening for their capabilities to reduce lipid content and thermal proteome profiling is applied to elucidate possible targets and mode of actions.; ii) Study the mode of action of endocrine disruptors compounds and possible input of its exposure to the development of metabolic disorders. We believe that this methodology could reduce considerable time and cost for assessment of multi-targeted drugs.

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## Short communication 15

# Histamine H4 receptors a new therapeutic target in postinfectious cardiomyopathy?

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Postinfectious cardiomyopathy in man often progresses to dilated cardiomyopathy, a major cause of chronic heart failure, disorder with a high morbidity and mortality, largely unmodified by a current treatment [1]. Histamine plays an important role in physiology of cardiovascular system[2]. Histamine is also well known mediator of inflammation and immune modulator, $H_4$ receptors ($H_4$Rs) being involved[3,4]. In this study we examined the effect of $H_4$Rs modulation on progression of experimental autoimmune myocarditis (EAM), the animal model of post infectious cardiomyopathy[5]. EAM was induced in male Lewis rats by immunization with porcine heart myosin in complete Freund's adjuvant (0.33 mg/s.c into each rear footpad), twice with one week apart[5]. Histamine $H_4$ receptor ligands, and Quinapril used as a reference[6], were applied daily either for the first 2 weeks of the 4 weeks lasting study or between 2 to 4 weeks post EAM induction. $H_4$R ligands employed: ST994 (N4-[4-methylbenzyl]-6-[4-methylpiperazin-1-yl]pyrimidine-2,4-diamine, neutral antagonist), ST1012 (4-[isoindolin-2-yl]-6-[4-methylpiperazin-1-yl]pyrimidin-2-amine, inverse agonist), ST1006 (N4-[2,6-dichlorobenzyl]-6-[4-methylpiperazin-1-yl]pyrimidine-2,4-diamine, partial agonist) were synthesized by German partner as described previously[7] and each was administered to rats by subcutaneously implanted Alzet osmotic pump, delivering 1mg drug/kg/day. Quinapril (20 mg/kg/day) was given by intragastric tube. Following myosin administration plasma ceruloplasmin activity, marker of inflammation, was at a high level with a peak at 2 weeks, the treated rats having it lower, the ST994 group significantly reduced. Transthoracic echocardiography performed before, and 2- and 3 weeks following a booster injection has shown the myocardial performance by the treated rats was better than that of untreated, even one week after the drug discontinuation. The decrements of FS (%) and LVEF (%) were much less FS: 14% and 51% while LVEF: 30% vs 57% for ST994-treated vs EAM-untreated, respectively. The functional improvement was associated with a lower mortality, smaller increase in a heart to body weight ratio, with histological evidence of a smaller deposition of interstitial collagen as revealed by Masson trichrome staining and lower myocardial mRNA expression of profibrotic cytokines TNF alpha and MCP-1. The attenuation of cardiac fibrosis was supported by the lower tissue hydroxyproline content. The benefits of H4R antagonist ST994 was further substantited when the treatment started 2 weeks after immunization. In this group only 5/9 rats completed the study; the mortality of other drug treated and EAM untreated amounted over 90%.

The results indicate histamine $H_4$ receptor modulation alleviate cardiac remodeling, preserve cardiac function and increase survival in rats with autoimmune myocarditis and may be of clinical relevance.

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## Short communication 16

# Molecular modeling of novel MAO-B/AChE dual inhibitors as potential anti-Parkinson agents

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It has become increasingly clear that the major basic processes involved in the neurodegenerative disorders, such as Alzheimer's (AD) and Parkinson's (PD) diseases, are multifactorial and although each disease has its own molecular mechanisms, some general pathways might be recognized in different pathogenic cascades. They include protein misfolding and aggregation, oxidative stress, free radical formation and mitochondrial dysfunction.[1] In this scenario, new 4-(3-nitrophenyl)thiazol-2-ylhydrazone derivatives were proposed as monoamine oxidase B (MAO-B) and acetylcholinesterase (AChE) dual inhibitors.[2] Rational molecular design, and targets recognition have been evaluated by means of molecular modelling. Compounds were synthesized and *in vitro* tested against MAO-B and AChE, and their corresponding isoforms, monoamine oxidase A (MAO-A) and butyrylcholinesterase (BuChE), respectively. Among the evaluated compounds, three inhibitors may be considered as promising dual inhibitors of MAO-B and AChE.

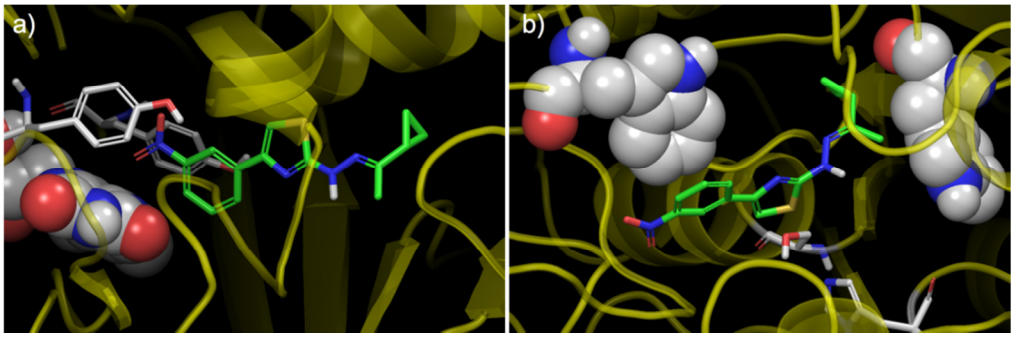


**Figure 1.** The most promising inhibitor binding modes in (a) MAO-B and (b) AChE active sites.

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## Short communication 17

# TAU-directed polypharmacological approach in Alzheimer's disease: development of a small library of thiazolidinediones

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Alzheimer's disease (AD) is the most common cause of dementia and the most prevalent neurodegenerative disorder. The current lack of effective drugs, with a drug failure rate of 99.6%, has led medicinal chemists to explore innovative therapeutic approaches to combat such a complex and multifactorial disorder.[1] Recent findings suggest that altered tau network is more significant than β-amyloid one from a pathogenic point of view. Particularly, from a pharmacological point of view, two points of intervention have been envisaged: inhibition of phosphorylating tau kinases (i.e. GSK-3β), and inhibition of tau aggregation process.[2] Thus, our aim is to restore the imbalanced network by acting on both hubs, through new small molecules able to simultaneously inhibit GSK-3β and tau aggregation process. Starting from a knowledge-based approach, we designed a small library of 2,4-thiazolidindione-derivatives (**1**-**36**). Their synthesis was achieved through an optimized, green, and solvent-free Knoevenagel condensation. Then, we set-up a screening pipeline for the identification of the best dual inhibitors (Fig 1). As all the compounds were not toxic, they were tested for their ability to inhibit GSK-3β. Then, the active compounds were assessed for their ability to cross the blood brain barrier, and for their neuro- and hepato-toxicity. Importantly, these assays allowed us to select the 5 best-performing compounds (**12**, **13**, **21**, **26**, **28**), which were evaluated for their ability to inhibit the tau aggregation process on the tau-derived hexapeptide AcPHF6 as a simple, fast and already validated model for drug discovery purposes.[3] Antiaggregating activity of compound **28** was also confirmed in a more clinically relevant model of tau aggregation, i.e. the recombinant human tau construct K18. Finally, anti-tau activity was demonstrated in the okadaic acid-induced tau hyperphosphorylation cell model.

For the first time, a polypharmacological drug discovery approach aimed at the discovery of novel small molecules able to inhibit the tau cascade in two different hubs has been effectively pursued.

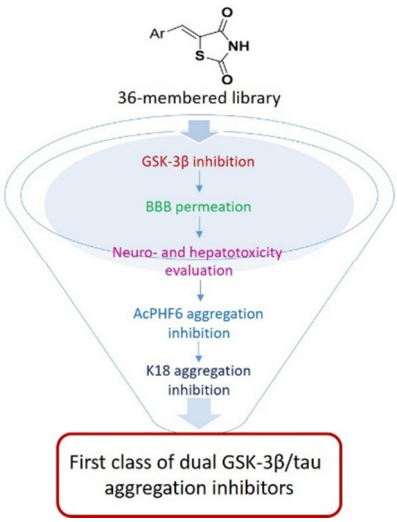


**Figure 1.** Screening pipeline.

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## Short communication 18

# Development of a plant genetic toolbox for the production of pharmaceutically-relevant triterpenes

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Plants have evolved a colossal arsenal of secondary metabolites to help them withstand environmental challenges. Humans may in turn benefit from these diverse and biologically active molecules to fight diseases and to contribute to the medicine of tomorrow. Among them, triterpenes represent one of the most structurally diverse classes of natural products, which present an astonishing array of biological activities. They are notably seen as multi-target therapeutic agents for the prevention and treatment of metabolic and vascular diseases[1]. In *Malus domestica* Borkh., triterpenes may be conjugated with hydroxycinnamic acids, which have been shown to increase triterpene biological efficacy in several health-related studies[2]. The lack of efficient and sustainable production system has however hindered their commercial deployment, and in turn, human health benefits.

Our team aims therefore at developing plant metabolic engineering tools for a sustainable production of pharmaceutically relevant triterpene esters (**Figure 1**).

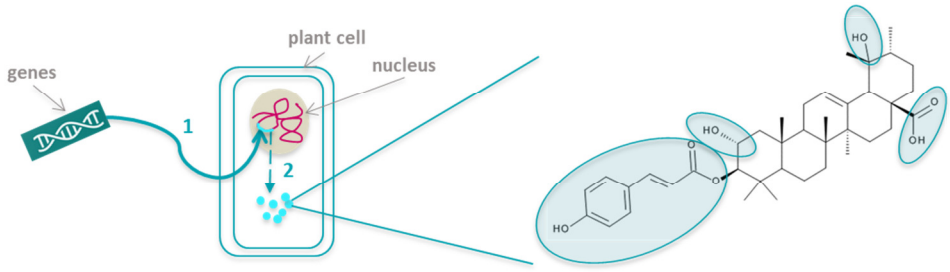


**Figure 1:** Schematic illustration of triterpene functionalization using plant genetic engineering

We currently tackle the development of a genetic toolbox constituted of decorating enzymes, regulators, and transporters for the rational design of triterpene esters. This knowledge will be of tremendous importance to develop plant-based systems able to produce high amount of functionalised triterpenes[3,4]. In particular, the potential of a promising and largely underexploited bio-based platform, i.e apple stem cell lines, is currently being unveiled using -omics technologies.

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## Short communication 19

# New efficient inhibitors of aldose reductase from the family of carboxymethylated indoles

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Aldose reductase inhibitors have been in the center of attention in drug design and discovery over the years. Although many potent ARIs have been identified, the majority of these also inhibit aldehyde reductase (ALR1), a related enzyme involved in the detoxification of reactive aldehydes. These findings are motivating for design of novel ARIs with attenuated toxicity and improved physicochemical profiles as potential therapeutic agents.Recently a series of new compounds with pyridoindole structure was synthesized and tested for the inhibition of rat lens aldose reductase. Structural modifications of the pyridoindole scaffold brought unexpected increase in efficiency. The most potent compound in this series, 2- (2-(ethoxycarbonyl)-8-methoxy-1,2,3,4-tetrahydro-pyrido [4,3-b] indol-5-yl), DPI-1, showed inhibitory activity more than 1000 times higher (IC50 = 12,6 nM), while being 780 times less active against rat ALR1 (IC50 = 9983 nM). It significantly decreased sciatic nerve and erythrocytes sorbitol levels in the streptozotocin (STZ) induces diabetic rat model (50 mg/kg/d for 5 days, p.o.). DPI-1 was found to inhibit efficiently reduction of the inflammation mediator glutathione 4-hydroxynonenal. DPI-1, the lead candidate of a new series of pyridoindole derivatives was identified as a highly effective and selective aldose reductase inhibitor with a promise of targeting long-term diabetic complications and inflammatory disorders related to lipid peroxidation.

The structural motif of the most efficient pyridoindole inhibitors, originally chosen from Chemspider database, is the base for combinatorial database developing actually in our institute.

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## Short communication 20

# Macrocyclic amidinoureas,

# a valid scaffold for antifungal and chitinase inhibiting compounds

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Systemic fungal infections are, nowadays, of crucial importance for patients affected by AIDS, that have been transplanted, or that are subjected to chemio/radiotherapy for tumors, situations that the immune system may result unable to manage, or because of the onset of resistance that, along with the fact that very few new classes of antifungals have been discovered, makes the research in this field of great importance and interest worldwide.

In the last decade, our group explored the great potential of natural and synthetic guanylated compounds, a great amount of work that led to the development of new non-azole macrocyclic compounds, endowed with antifungal activity, which have been deeply characterized through both *in vitro* and *in vivo* assays.

During the search for a putative target for these compounds, the scope of the macrocyclic amidinoureas has been widened, since they showed also anti-Chitinase activity. Chitinases resulted to be a hot topic recently, because of their involvement in many parasites life cycles and in human inflammatory pathologies, hence we rationally designed and investigated macrocyclic derivatives as Chitinase inhibitors, exploring the possibilities of this peculiar scaffold. Preliminary *in vitro* assays against *T. viride* Chitinase have been performed, leading to the identification of a promising HIT compounds to be further improved, with a 50-fold improvement in terms of $K_i$ if compared to the previously tested compounds.

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## Short communication 21

# Efficient virtual screening and prediction of binding profiles

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Frustrated by the poor reliability and performance of molecular docking software tools and scoring functions associated to them led us into development of a novel virtual screening (VS) tool that fuses the protein structure-based methods with ligand-based methods [1]. In addition to the standard VS-mode, we have recently implemented fragment- and pharmacophore-based drug discovery strategies into our VS protocol. Also, the post-processing of molecular docking results with our method enables us to enrich the identification of active ligands. The major advantages of our method are speed and accuracy, especially when compared to the widely used docking software tools. Furthermore, based on our VS efforts we have designed and synthesized a library of small molecules with the same core-structure. This library contains both potent&selective molecules for each of these targets and molecules with more varied selectivity profiles. Our main targets are cancer-related protein-protein interactions and targets that are associated to synthesis and binding of estradiol, such as 17beta-hydroxysteroid dehydrogenase 1, aromatase, estrogen receptor [2], and several enzymes typically involved in the metabolism of such compounds [3].

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## Short communication 22

# Novel techniques for multitarget and multi-antitarget design for blebbistatin analogs

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In multi-target drug design, it is important to distinguish actives and inactives from among a variety of targets and anti-targets. Indeed, multi-target strategies test the limits of available technology, be that in testing a large number of compounds versus a large number of targets, in silico methods for dealing and understanding these relationships, and predicting reliably pharmacological outcomes.[1,2] In the case of blebbistatin analogs,[3] used to inhibit myosin in the treatment of motility diseases, standard and augmented structure-based design techniques could not recover the observed profiles of compounds. A ligand-based method was able to select those compounds which were active. This method was also used to predict toxicity through androgen receptor binding, using an approach that included Bayesian naïve classifiers, docking scores of binders and non-binders, as well as chemical fingerprints, in a multivariate logistic regression. PAINS (non-specific, assay-interfering compounds) and reactive groups, as well as anti-targets,[4] were also evaluated through models.

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## Short communication 23

# Self-assembled ligands targeting TLR7: a molecular level investigation

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Toll-like Receptors (TLRs) are pattern recognition transmembrane proteins that play an important role in innate immunity. In particular, TLR7 plays a role in detecting nucleic acids derived from viruses and bacteria. The huge number of pathologies in which TLR7 is involved has led to an increasing interest in developing new compounds targeting this protein. Several conjugation strategies were proposed for TLR7 agonists to increase the potency while maintaining a low toxicity. In this talk, we present some results obtained by molecular dynamics (MD) on two promising classes of TLR7 compounds derived from the same pharmacophore conjugated with phospholipid and polyethylene glycol (PEG), respectively. MD confirmed dynamic light scattering (DLS) measurements showing how only the phospholipid conjugation provides the compound abilities to self-assemble in an orderly fashion with a maximal pharmacophore exposition to the solvent. Further EPR and cytotoxicity experiments highlighted that phospholipid compounds organize in stable aggregates and well interact with TLR7, whereas PEG conjugation was characterized by poorly stable aggregates at the cells surface. The methodological framework proposed in this study may be used to investigate, at a molecular level, the interactions generally occurring between aggregated ligands and protein receptors

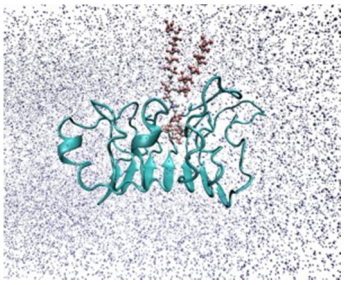

**Figure 1:** Toll-like receptor 7 modulators

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## Short communication 24

# Induced Fit Docking Protocol applied to the *in silico* evaluation of antiviral HIV-1 integrase resistance

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HIV-1 Integrase (IN) is an essential enzyme for HIV-1 replication since it catalyzes the integration of viral DNA into the host cell-genome. IN strand transfer inhibitors Raltegravir (RAL), Elvitegravir (EVG) and Dolutegravir (DTG) represent the most recent and long-awaited addition to the arsenal of drugs to treat HIV-1 infections.[1] DTG, belonging to the second generation of IN inhibitors, efficiently inhibits integration of the wild-type (WT) virus, but also the viral strains resistant to RAL and EVG. DTG has a high genetic barrier since no pathway of resistance has been clearly identified. However, several studies reported that DTG is less efficient when used for RAL/EVG-experienced patients who had previously suffered from virological failure. In this study, the clinicians report the case of different mutations found in a patient previously treated with RAL, with a particular focus on the impact of N155H in terms of DTG susceptibility. Starting from PFV IN crystal structures, deposited in the Protein Data Bank (PDB),[2] our computational studies were performed. Docking studies were carried out with Schrödinger's Induced Fit Protocol (IFD)[3] in order to evaluate the impact of N155H substitution, alone and in presence of specific mutational patterns. Our results suggested that these mutations affect DTG binding affinity and could negatively modulate the virological success.

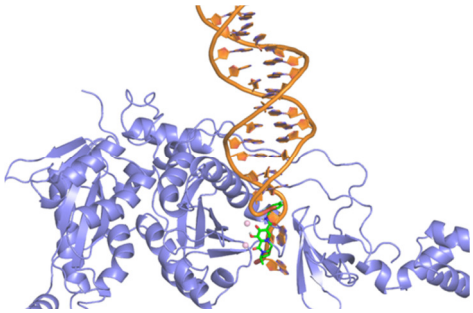

**Figure 1:** 3D viewer of DTG in PFV intasome catalytic binding site.

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## Short communication 25

# STD-NMR-*in silico* Screening: An Effective Combination for Validating ChE and MAO-B Dual Inhibition

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Unfortunately, despite the best efforts of all stake-holders involved, currently there is no cure for Alzheimer's disease nor does there appear to be any one soon. The figures are staggering, and it is expected that by 2030, the number of people with the disease will rise to more than 70 million worldwide.[1] Multiple factors contribute to the pathology and the disease process seems to involve several cellular and molecular aberrations.

The only current solution for sufferers is treatment of the symptoms, is the use of prescribed drugs such as Donepezil, Rivastigamine and Galantamine, which are potent Cholinesterase inhibitors. Monoamine oxidase-B (MAO-B) inhibition has also been recognized as an alternative therapeutic approach, as studies confirm that it confers important neuroprotective properties.[2]

Our group has an on-going program developing and screening novel dual ChE and MAO-B inhibitors. In order to improve the efficacy of our screening program for the development of more efficacious leads, we use a dual STD (Saturation Transfer Difference)-NMR/*in silico* (ligand docking approach) to refine our hits. This approach provides an effective validation method in the search for new dual inhibitors for key AD targets. Some of our case studies on the development of dual-inhibitors for ChE and MAO-B will be discussed in this communication.

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## Short communication 26

# Multi-level strategy for analysis of bioactive drug conformations

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Exploring the conformational preferences of small flexible ligands plays an increasingly important role in drug design. Estimating the relative free energy of a ligand in its target-bound state (i.e. the bioactive conformation) is necessary to understand the process of molecular recognition, to optimize the potency of bioactive molecules and to improve the accuracy of structure-based drug design methods. [1, 2] A set of 100 crystal structures of pharmaceutically relevant drug-like molecules was tested using multi-level computational strategy. [3] We combined low-level (LL) method for sampling the conformational minima and high-level (HL) ab-initio calculations for estimating their relative stability. [1] The method was automated and tested on various ligands with different numbers of atoms, charge and rotatable bonds. The analysis show that is necessary to perform Hamiltonian Replica Exchange simulations in order to explore all possible states of energy landscape of given dihedrals. Our findings suggest that the method is an effective way to improve the conformational sampling of the drug-like molecules. In the most cases, we found that the cluster representatives of the ligands have less than 1.0 A° RMSD difference with respect to the bioactive conformation bound in complex. Moreover, our quantum mechanical results report that the bioactive conformation is around 2 kcal/mol higher in potential energy than the lowest-energy conformation. Taking into account the flexible nature of molecules, protonation state and tautomeric forms, make our task even more challenging. The proposed strategy may represent an efficient tool for predicting the conformational landscape of drugs while keeping a reasonable balance between chemical accuracy and computational cost. The multilevel strategy was tested on more than 100 pharmaceutically relevant complexes and the whole data set with analysis is available on the Bioactive Compounds webserver. It is a web-based tool for the for analysis of bioactive drug conformations and the flexibility of ligands in solution. The server incorporates powerful protocols for prediction of 3D bioactive conformations or ensemble of potential bioactive conformations for known and novel drugs.

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# Poster communications

## Poster communication 1 – WG 2

# Ruthenium(III) complexes containing Schiff base ligands with promising anticancer activity

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Schiff bases and their metal complexes are attractive to the scientific community because of their potential ability to react with different cellular/molecular targets and show impressive biological activities including antitumor properties. On the other hand, the promising antineoplastic potential of various ruthenium compounds has also been reported [1].

The aim of our study was to evaluate the effect of newly synthesized ruthenium(III) complexes with Schiff bases resulted from the condensation reaction between salicylaldehyde and ethylenediamine (Salen), 1,3-diaminopropane (Salpn) and 1,2-phenylenediamine (Salphen), respectively on cell viability and proliferation. The following human cell lines were used as model systems in our study: MDA-MB-231 (triple negative breast cancer), HeLa (cervical carcinoma) and Lep-3 (non-tumor embryonic fibroblasts). The investigations were performed on monolayer cell cultures with short-term experiments (24 – 72 h) using thiazolyl blue tetrazolium bromide test - MTT test, neutral red uptake cytotoxicity assay, crystal violet staining and double staining with acridine orange and propidium iodide. Long-term experiments (25-30 days) were carried out to assess the influence of the compounds on 3D cancer cell colony formation.

The obtained results reveal that applied at ~~a~~ concentrations in the range 5-100 μg/ml the investigated complexes decrease the growth and viability of the treated cells in a time- and concentration-dependent manner; Ruthenium(III) complexe~~s~~ with Salen has been found to be the most promising cytotoxic/cytostatic agent; cancer cells (MDA-MB-231, HeLa) are more sensitive to the cytotoxic effect of the compounds as compared to non-tumor Lep-3 embryonic fibroblasts.

Key words: Metal complexes, Schiff bases, Ruthenium(III), Cancer, Cell lines, Cytotoxic activity

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## Poster communication 2 – WG 4

# *In silico* approaches for antiviral Multi-target drugs discovery

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The rational design of dually active inhibitors against human immunodeficiency virus (HIV) reverse transcriptase and integrase represents an important goal for improving the clinical management of acquired immunodeficiency syndrome (AIDS). HIV-1 reverse transcriptase (RT) and integrase (IN) are essential enzymes for the virus replication, since the first catalyzes the conversion of virus single-stranded RNA genome to double stranded DNA and the second the integration of the viral DNA into the host cell-genome. HIV-1 RT has two associated functions: the RNA- and DNA-dependent DNA polymerase function and the ribonuclease H (RNase H) function.[1] RNase H and IN are validated targets for dual inhibitors drug development, due to their structural similarities, since their catalytic binding site contains conserved DDE triad and a pair of divalent $Mg^{2+}$/$Mn^{2+}$ ions.

Starting from the RT and IN crystal structures, deposited in the Protein Data Bank (PDB),[2] our computational studies were performed. Docking simulations were carried out by using Glide Standard Precision (SP) protocol of Glide v.7.2.[3], with the aim to perform a structure-based virtual screening of different database compounds, thus finding new potential dual inhibitors against HIV-1 IN and RT RNase H.

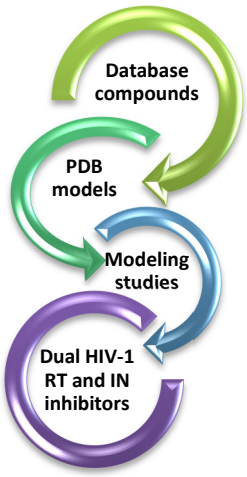


**Figure 1:** *In silico* approaches in Multi-target drug discovery.

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## Poster communication 3 – WG 2

# ASSESSMENT OF MECHANISMS OF TOXICITY OF NAPHTHOQUINONES THROUGH A YEAST-BASED APPROACH.

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The quinone moiety is frequently found in natural products obtained from plants and microorganisms. Many of these products possess strong antimicrobial and antiparasitic activities and some have been introduced in the clinic to treat diseases such as cancer and malaria. Quinones may exert their biological activities through three basic mechanisms: (i) reactivity against cell nucleophiles; (ii) one or two electron redox cycling in the presence of dioxygen; and (iii) inhibition of key enzymes or structural proteins such as topoisomerase II, protein tyrosine phosphatases, tubulin, etc.

The yeast *Saccharomyces cerevisiae* is particularly suited to study chemical-biological interactions of quinones because of its unique ability to perform aerobic fermentation and grow in the absence dioxygen and mitochondria.[1] We have used these special features to characterize the mechanism of toxicity of several natural and synthetic naphthoquinones related to lawsone, lapachol, juglone and β-lapachone. We have found we can classify yeast cytotoxic naphthoquinones in three groups in terms of mechanism of toxicity. The first group comprises quinones whose toxicity greatly depends on dioxygen, exert also a strong oxidative stress to the cell, and selectively target mitochondrial function. The second group comprises quinones which shared with the first group the generation of oxidative stress but fail to target mitochondria significatively. The third group includes quinones whose cytotoxicity goes beyond any involvement of dioxygen, oxidative stress or mitochondrial function. Surprisingly, even minor semisynthetic modifications in the products are able to change the mode of cytotoxicity for the studied quinones.

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## Poster communication 4 – WG 4

# Identification of AChE/MAO-B multi-target directed ligands using a dynamic pharmacophore model approach

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It has become increasingly clear that the major basic processes involved in the neurodegenerative disorders, such as Alzheimer's (AD) and Parkinson's (PD) diseases, are multifactorial and although each disease has its own molecular mechanisms, some general pathways might be recognized in different pathogenic cascades. They include protein misfolding and aggregation, oxidative stress, free radical formation and mitochondrial dysfunction.[1] In this scenario, our goal was to discover, by computational methods, synergistic inhibitors of both Acetylcholinesterase (AChE) and Monoamino oxidase B (MAO-B), restoring the neurotrasmitters bioavailability and reducing oxidative stress due to the MAO-B catalytic activity. With the aim to better elucidate the ligands effects on protein active conformations, we considered for each target a number of crystal structures containing different co-crystallised ligands. Theoretical target models were submitted to 100 ns of molecular dynamics simulation sampling 1000 conformers at regualt time intervals equal to 100 ps. For each sampled frame, using LigandScout software,[2] a pharmacophore model was computed.[3] Such a dynamic approach highlighted ligand-protein interactions and suggested key features to design a AChE/MAO-B dual inhibitor pharmacophore model.

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## Poster communication 5 – WG 4

# Dendrimers as drug carriers. Insight of host-guest interactions by Molecular Dynamics Simulations and NMR spectroscopy

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PAMAM dendrimers are synthetic macromolecules with unique structural properties, such as nanoscale and well-defined size, monodispersity and hyperbranched structures that possess hydrophobic inner cavities and multivalent surface functionalities.[1] Due to these interesting properties these dendrimers have attracted the attention of the scientific community as promissory drug carriers for biomedical applications. As drug carriers, PAMAM dendrimers have shown improvements in the pharmacokinetic and pharmacodynamics properties of several drugs, allowing the reduction of drug dosages and unwanted side effects, which is especially relevant for anticancer drug delivery. [2]

In this work, we present a complementary theoretical-experimental study about the host-guest interactions between PAMAM dendrimers and chemically diverse drugs, which incluying anticancer and nonsteroidal anti-inflammatory drugs NSAIDs. Fully atomistic molecular dynamics (MD) simulations in combination with 2D-NOESY NRM experiments and absorption spectroscopy were employed to examine the host-guest chemistry of PAMAM-DRUG complexes (Figure 1). Parameters as complex stoichiometry, binding constat ($k_b$), preferential binding sites and non-bondindg interactions between dendrimers and drugs were stimated, finding a good theoretical-experimental agreement. [3]

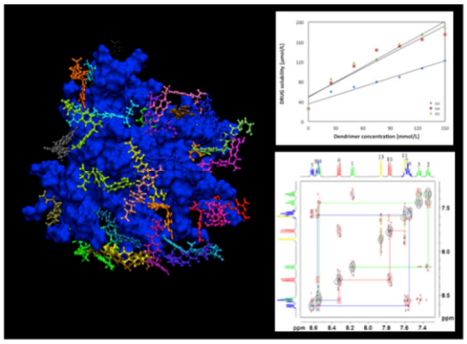

**Figure 1:** Drug-Dendrimer complexes and its host-guest interactions.

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## Poster communication 6 – WG 1

# Design, synthesis and biological activity studies on novel tacrine-donepezil hybrides as MTDL against Alzheimer's disease

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Alzheimer's disease (AD), the most common form of demantia in the elderly people, is a neurodegenerative disease chracterized by various pathologic patways including decreased acetylcholinesterase/butyrylcholinesterase (AChE/BuChE) activity, formation and accumulation of toxic amyloid-β (Aβ) plaques, defects in tau protein phosphrolation.[1]

Considering the multifaceted nature of the AD pathogenesis, targetting more than one threapeutically active site is getting more and more attention in the recent years to design novel scaffolds for the treatment of AD.[2]

There are four approved AChE inhibitors for the symptomatic treatment of AD: Tacrine, donepezil, rivastigmine, and galantamine. Although, tacrine is no longer used in the treatment of AD due to its hepatotoxicity, it is still a widely used scaffold in the design of Multi Target-Directed Ligands (MTDLs) thanks to its high affinity to AChE.[2] Donepezil, the first choice medication in the treatment of AD, is a dual inhibitor of AChE with its ability to bind both catalytic active site and peripheral anionic site of the enzyme, simultanuously.[3]

We have reported that hydrazone containing molecules are privilaged structures against both AChE/BuChE inhibition and inhibition of Aβ formation in our previous studies.[4]

In this study, we chose the benzylpiperidine moiety of donepezil and tacrine as core structures and connected them with hydrazone functinal group to aim dual inhibition of AChE. We designed and synthesized novel substituted 9-hydrazinyl-1,2,3,4-tetrahydroacridine derivatives and tested them against AChE/BuChE inhibitory activity, inhibition of Aβ formation and additionally, the ability to form metal complex.

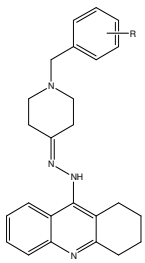


**Figure 1:** Structure of the title compounds.

The synthesis of the title compounds realized in 4 steps. In the first step, anthranilic acid and cyclohexanone were reacted with $POCl_3$ to obtain 9-chloro- 1,2,3,4-tetrahydroacridine.[5] Aromatic nucleophilic substitution of 9-chloro-1,2,3,4-tetrahydroacridine with hydrazine hydrate gave 9-hydrazino- 1,2,3,4-tetrahydroacrine. Substituted benzylpiperidinone moiety was synthesized as donepezil-like moiety. Lastly, substituted benzylpiperidinone was reacted with hydrazine derivative to obtain novel hydrazone-containing compounds.[6]

The inhibitory activitiy of the target compounds against AChE and BuChE were evaluated by slightly modified Ellman's method. Inhibitory activity of the title compounds was calculated by means of $IC_{50}$ values and the final compounds exhibited good inhibitory activity.[7] Additionally, final compounds were evaluated for β-amyloid formation inhibition

according to Thioflavin-T test and title compounds exhibited inhibitory potency towards β-amyloid formation.[8] The ability to form metal ion complex of the final compounds with biometals such as $Zn^{+2}$, $Fe^{+2}$, $Cu^{+2}$ was investigated.[9]

In conclusion, we designed, synthesized and characterized tacrine-donepezil hybride molecules as dual inhibitors of ChE. According to the bioactivity results of our study, this scaffold is a promising structure as a MTDL in the treatment of AD and further researches are ongoing enthusiastically on this scaffold in our laboratory.

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## Poster communication 7 – WG 1

# Withaferin A-Silyl Ether Analogues as Multitarget Ovarian Apoptotic Inducers

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Ovarian cancer (OC) represents the seventh most commonly diagnosed cancer worldwide. Nearly 70% of patients suffer a relapse within 6 months of the last chemotherapeutic cycle, which is attributed to patients eventually develop resistance to carboplatin and paclitaxel adjuvant chemotherapy.[1] Thus, there is an urgent need for new therapies to improve the prognosis of OC for patients who relapse. Whitaferin A (WA) is one of the main biologically active whitanolides, exerting a wide range of biological properties, including *in vitro* and *in vivo* cell proliferation suppression by apoptosis induction.[2] Molecular events associated with WA-induced apoptosis involve multiple key cell-survival and regulatory pathway, making WA a promising multitarget compound for future drug development. Previously, we reported that incorporation of silyl substituents in the WA-framework enhance its cytotoxic effect and induction of apoptosis by the organosilicon analogues.[3] Therefore, encouraged by this previous work, we continue our efforts to enlarge the chemical space of WA to improves its clinical potential as anticancer agent.

The current study reports the synthesis and evaluation of WA-silyl ethers analogues with enhanced cytotoxicity on human ovarian carcinoma cells (A2780 and A2780-CP70, a cisplatin-resistant cell line). Some analogues exhibited $IC_{50}$ values in the nanomolar range, with 27-*O*-(tripropylsilyl)-WA being the most promising analogue on the cisplatin-resistant cell line ($IC_{50}$ 3.2 nM). Furthermore, selected analogues were investigated for their ability to induce apoptosis.

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## Poster communication 8 – WG 1

# Cationic derivatives based on natural scaffolds as dual-target agents for Alzheimer's disease

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The Alzheimer's disease (AD) is multifactorial and neuronal death is the result of a complex network of pathological stimuli over an extended period of time. Current single-target treatments are only palliative and fail to modify disease progression. 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. Thus, one way to prevent or retard the progression of oxidative damage is the regulation of ROS production in mitochondria by exogenous antioxidants. In this context, the innovative mitochondria-targeted antioxidants based on naturally occurring caffeic acid were developed. The resulting compounds were optimized from the TPP-based AntiOxCIN4 and AntiOxCIN6 lead compounds by insertion of different smart cationic careers, such as 4-picoline, isoquinoline and imidazole.

The results obtained showed that the synthetized compounds presented inhibitory activity on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) isoforms. In addition, most of novel antioxidants did not induced cytotoxicity in neuroblastoma SH-SY5Y cells and simple chemical modification in cation carrier yield derivatives that can prevent hydroxydopamine (6-OHDA), FeNTA and hydrogen peroxide ($H_2O_2$) induced damage. On the other hand, the type of cationic carrier has also influence the antioxidant potency outline. The great potential of novel mitochondria-targeted antioxidants will lead to further optimization processes to achieve a valid therapeutic strategy to prevent or delay the deleterious oxidative stress effects.

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## Poster communication 9 – WG 1

# Hydroxycinnamic acid derivatives and sulfured analogues: from synthesis to antioxidant properties

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Hydroxycinnamic acids (HCAs), an important class of polyphenolic compounds, have multiple activities with biomedical interest (e.g. antibacterial, antiviral, anti-inflammatory, neuroprotective) which have been ascribed to their antioxidant activity.[1] Over the last decade, several modifications of natural scaffolds have been performed to obtain new antioxidants with improved pharmacological properties.[2] These approaches include, among others, the replacement of the hydroxyl group (OH) by a sulfhydryl group (SH) [3] and the introduction of lipophilic substituents.[4]

The goal of the present work is the design and development of new HCA derivatives (**Figure 1**) endowed with neuroprotective activity.

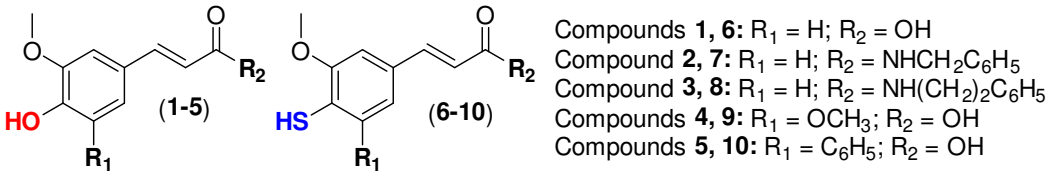


**Figure 1**: Chemical structure of hydroxycinnamic acid derivatives.

HCA-based antioxidants were successfully obtained. The antioxidant activity of the synthesized compounds was assessed by total antioxidant capacity assays, namely DPPH$^{\bullet}$, ABTS$^{\bullet+}$, galvinoxyl and ORAC methods, and the evaluation of the redox potential by differential pulse voltammetry. Cell-based assays were performed in SH-SY5Y neuroblastoma cells to evaluate the cytotoxicity of the compounds as well as the neuroprotection profile against 6-hydroxydopamine. The results obtained so far will be presented in this communication.

Acknowledgements: This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980, and NORTE-01-0145-FEDER-000028). D. Chavarria (SFRH/BD/108119/2015) grant was also supported by FCT and FEDER/COMPETE funds.

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## Poster communication 10 – WG 1

# Synthesis of *trans*-fused oxacycles by means of Prins cyclization

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Many Marine Natural Products are based in scaffolds which cannot be found in terrestrial products.[1] A very good example of these structures is the Brevetoxin B (**1**), first isolated from the *Gymnodinium breve* by the Nakanishi group in 1981.[2] The core of this marine ladder toxin is composed by eleven diverse sized *trans*-fused oxacycles.

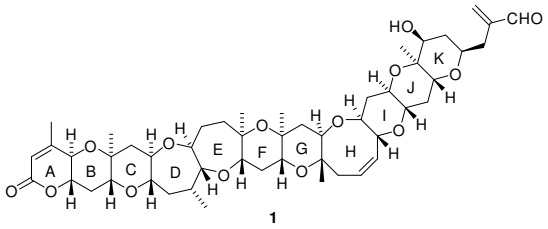


**Figure 1:** Structure of Brevetoxin B.

In our research group we have used the Prins cyclization, catalyzed by iron(III) salts,[3] as the main tool to obtain this type of *trans*-fused oxacycles in a very straightforward manner.

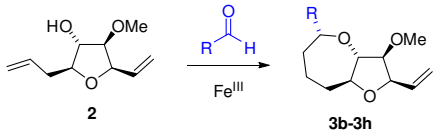


**Figure 2:** Obtention of *trans*-fused oxacycles.

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## Poster communication 11 – WG 2

# Zoanthamine-like alkaloids as possible agents in the treatment and prevention of osteoporosis.

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Osteoporosis is a disease in which the density and quality of bone are reduced, increasing the risk of fracture. The incidence of fractures increases with age beginning at about 50. In Europe, the size of the population with 50 o more years with highest risk, is expected to increase by 26% in women and 36% in men for 2050. The projected increase in the ageing population will lead to an increasing frail population at greater risk of falls and fractures. We study a lead compound that shows a novel mechanism of action by stabilizing collagen with the following objectives: i) To open a novel path for a rational design of active compounds. *ii)* To develop a device for controlled release of bone regenerating molecules in osteoporotic patients. In this communication, we will show results of computational simulations and spectroscopic measurements of the interaction between norzoanthamine and collagen, a protein that is essential for the bone and cartilaginous structures of the body.

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## Poster communication 12 – WG 1

# Integrative Pericyclic Cascade: Strategy for the Construction of Molecular Complexity

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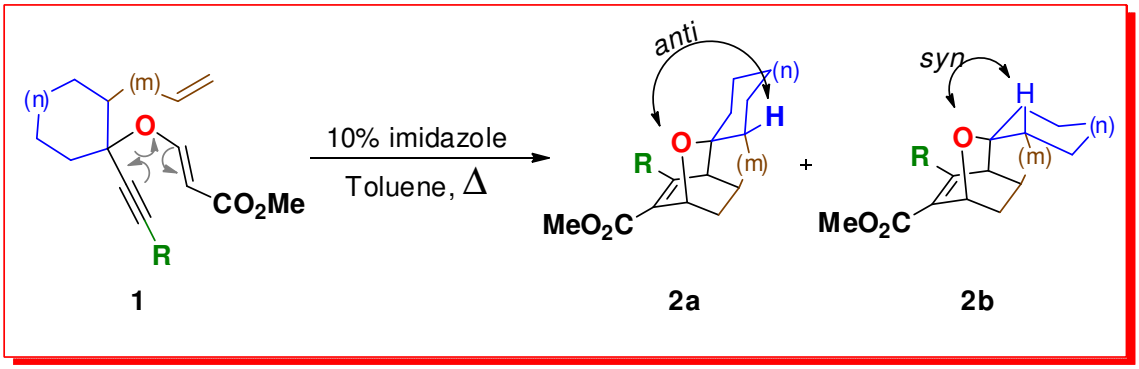
An all-pericyclic manifold is developed for the construction of topologically diverse, structurally complex and natural product-like polycyclic chemotypes. The manifold uses readily accessible tertiary propargyl vinyl ethers (**1**) as substrates and imidazole as a catalyst to form up to two new rings, three new C-C bonds, six stereogenic centers and one transannular oxo-bridge (**2a**/**2b**). The manifold is efficient, scalable and instrumentally simple to perform and entails a propargyl Claisen rearrangement–[1,3]H shift, an oxa-6π-electrocyclization, and an intramolecular Diels–Alder reaction.

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## Poster communication 13 – WG 2

# Mitochondria-directed antioxidants increased metabolic viability of human skin fibroblasts from Parkinson's disease patients

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Parkinson's Disease (PD) is a neurodegenerative disorder affecting more than 10 million people worldwide. Currently, PD has no cure and no early diagnostics method exist. Mitochondrial dysfunction in different PD models has been demonstrated and is considered an important pathophysiology component. Consequently, mitochondria are a relevant target for pharmacological interventions and the selective inhibition of mitochondrial oxidative damage is in fact a promising therapeutic strategy. Mitochondria-targeted antioxidants have been studied as an effective intervention to block mitochondrial oxidative damage and improve mitochondrial function. The role and beneficial effects of natural antioxidants, in particular cinnamic acids, have being largely studied due to the potent antioxidant activity exhibit throughout different mechanisms, anticipating beneficial effects in diseases with an oxidative component, including neurodegenerative disorders.

Mitochondria-targeted antioxidants based on cinnamic acids (AntiOxCINs) were developed by attaching the natural hydrophilic antioxidant caffeic acid to an aliphatic lipophilic carbon chain containing a triphenylphosphonium (TPP) cation. The antioxidant efficacy in mitochondrial systems was already confirmed by us. Our long-term objective was to evaluate whether mitochondria-targeted antioxidants (AntiOxCINs) could be used to improve the impaired metabolic phenotype found in PD. We used human skin fibroblasts collected from PD patients, as minimally invasive tool, to characterize the preexisting-metabolic and mitochondrial imbalance and to test the possible therapeutic efficacy of AntiOxCINs.

Human skin fibroblasts from PD patients revealed a reduced metabolic viability with decreased mitochondrial function, lower mitochondrial polarization and ATP levels concomitant with increased mitochondrial oxidative stress and SOD2 activity, when compared to age-matched controls. Treatment of PD fibroblasts with AntiOxCINs, with non-toxic concentrations and during 72 h, ameliorated the mitochondrial function and turned the PD fibroblasts physiologically more similar to their matched controls.

Our data points out the importance of developing mitochondriotropic antioxidants based on dietary scaffolds and the clarification of their cellular mechanisms. In this sense, further work will allow to identify similar molecules that can act as therapeutic agents for oxidative stress-related diseases, namely PD.

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## Poster communication 14 – WG 2

# Bioprospecting of marine bacteria from the submarine volcano Tagoro

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The extreme physical-chemical conditions of the oceans have favored marine organisms to produce a great variety of molecules as a mechanism to ensure their survival. These compounds are unique and represent a reservoir for the discovery novel bioactive compounds of great biopharmaceutical potential.[1] In particular, the marine microbiota, such as bacteria, cyanobacteria, yeasts, fungi and microalgae, represents a promising and inexhaustible source for the development of new drugs.[2]

In October 2016, we participated in the oceanographic campaigns Vulcano1016, as part of the Vulcano-II project (CTM2014-51837-R),[3] a multidisciplinary project that studies, from different perspectives, the recent submarine volcano Tagoro, created out of the volcanic eruption in El Hierro Island (Canary Islands), between late 2011 and early 2012. The eruption of Tagoro, which caused a severe impact on the marine life, stimulated the bacterial activity at the same time. Five years later, our involvement in the Vulcano1016 campaign was a unique opportunity to access a new micro-ecosystem under development with the main objective of isolating endophytic or associated microorganisms with both rock samples and deep-sea invertebrates.

In this paper we present the preliminary results of the taxonomic study of the samples taken from the new volcano Tagoro and from the protected marine area of La Restinga, which has allowed us to generate a collection of more than 120 strains of marine bacteria. These bacteria are under study to identify novel strains of interest for their antiproliferative activity.

**Acknowledgments:** We thank support of Dr. E. Fraile-Nuez (IEO), IP of the Vulcano-II Project (CTM2014-51837-R), Cabildo de Tenerife and PharmaMar, S.A. within the framework of the Agustín de Betancourt 2016 Program, the financing of project CTQ2014-55888-C03-01 / R (MINECO) and CONACYT, by grant number 504923/290666. Authors thank J. Manuel Padrón for the antiproliferative tests.

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## Poster communication 15 – WG 1

# Synthesis and evaluation of novel chalcones as dual antimicrobial/anti-inflammatory agents

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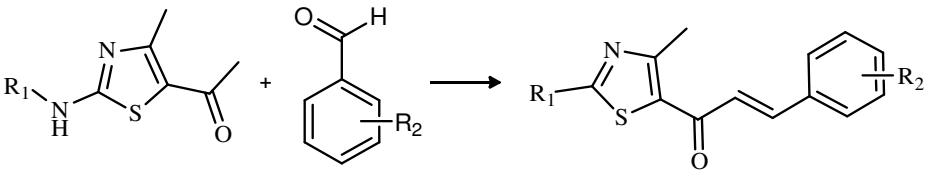
The increasing resistance to existing antimicrobial treatment has resulted in urgent demand for new classes of antimicrobial agents with different modes of action that could target both sensitive and resistant strains.[1] This need is even greater for patients suffering from chronic inflammatory bowel diseases. During an inflammatory response in the gut, some commensal microorganisms such as *E.coli* and *C.albicans* can thrive and contribute to disease.[2]

Chalcone analogs are known to possess antimicrobial, as well as anti-inflammatory activity, among a wide spectrum of biological activities. Taking also into account that many thiazole derivatives exhibit both actions, a series of 13 novel chalcones containing thiazol-5-yl moiety were designed and synthesized. The synthesis and general structure of the compounds are presented in Scheme 1.




Scheme 1. Synthesis of novel chalcones

The antimicrobial activity was assessed by microdilution method. The compounds were tested against bacteria (sensitive and resistant strains) and fungi. As reference drugs were used a) ampicillin, streptomycin and b) ketoconazole, bifonazole for the antibacterial and antifungal assays respectively. Furthermore, all newly synthesized compounds were tested for their anti-inflammatory activity by carrageenan mouse paw edema model, using indomethacin as reference drug.

The novel chalcones exhibited remarkable antibacterial and antifungal activities, being in every case more potent than reference drugs. They also exhibited considerable anti-inflammatory activity. These promising results are leading the way for further investigation in order to clarify the mode of action at molecular level, responsible for the activities observed.

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## Poster communication 16 – WG 1

# Microbiological activity of metal complexes of bis-1,8-naphthalimides

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Metal complexes of some organic ligands exhibit an antibacterial effect against different types of Gram-positive or Gram-negative bacteria. Zn(II) and Cu(II) ions are an essential part of many living organisms having a significant role in all aspects of enzymatic and non-enzymatic metabolisms in the biological systems.

In the recent years considerable attention has been paid to the Zn(II) or Cu(II) complexes with organic ligands as a new generation of antimicrobial, antiviral, anti-inflammatory and antitumor agents. The intensive investigations in this field are due to the effectiveness of antibiotics has been increasingly compromised by the rise of drug resistance and multiple drug resistant bacteria, which have become a new clinically common and serious threat to public health. As a solution cyclic imides with large π-conjugated backbone derivatives of 1,8-Naphthalimide - a very important class of organic compounds with a different type of biological activity, including antibacterial, antiviral, analgesics and anticancer etc. - have been investigated. To reveal the drug potential of those compounds various aspects of the antibacterial and antifungal activity and the minimum inhibitory concentrations (MICs) against some pathogenic Gram-positive and Gram-negative bacteria and yeast strains of Zn(II) or Cu(II) complexesof bis-1,8-naphthalimide units have been determined and discussed. The relationship of biological activity has been discussed as dependent on the compounds chemical structure. The results suggest that the investigated 1,8-naphthalimide compounds could find application in designing new antimicrobial preparations to control the spread of infections.

**Acknowledgements**

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## Poster communication 17 – WG 4

# Mapping the conformational and structural profile of the human 20S proteasome to reverse inhibition resistance

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The Ubiquitin Proteasome Pathway (UPP) plays a pivotal role in intracellular protein degradation and turnover in eukaryotic cells.[1] Therefore, modulation of the UPP emerged as a rational therapeutic approach in cancer, neurodegenerative diseases (Alzheimer, Parkinson), inflammatory pathologies (arthritis, psoriasis, asthma, colitis), organ transplant, infective diseases (malaria), among others.[2]

During the last two decades academia and pharmaceutical industry made huge efforts to develop natural and synthetic proteasome inhibitors (PI). In 2003 FDA approved the pioneering dipeptidyl boronic acid derivative PI bortezomib for the treatment of refractory multiple myeloma (MM) and subsequently frontline therapy for MM. However, despite the enormous potential of PI, their use is still limited to certain types of blood cancer and shows severe side effects, dose limiting toxicity, peripheral neuropathy, limited activity in solid tumour and innate or acquired drug resistance.[3]

In this work, we have used Molecular Dynamics (MD) simulations to perform the first conformational and structural characterization of the human native 20S proteasome structure[4]. We focused our analysis on the three catalytic subunits well known for their proteolytic activity (β1, β2 and β5) and we further extended our study to additional MD simulations of three different point mutations in the β5 catalytic subunit, with recognized importance in PI's resistance: Ala49Thr, Ala50Val and Cys52Phe. Hopefully, our studies will be able to shed the light on the structural key determinants that regulate the observed PI's resistance in the different mutations, and ultimately use the acquired knowledge in the development of new alternative and efficient proteasome inhibitors.

*Acknowledgements:* We thank the Fundação para a Ciência e a Tecnologia for financial support PTDC/QEQ-MED/7042/2014, UID/DTP/04138/2013, Mutalig Cost action and SAICTPAC/0019/2015.

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## Poster communication 18 – WG 2

# Profiling of known drug libraries against therapeutically relevant molecular targets

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The Fraunhofer Institute for Molecular Biology & Applied Ecology (IME) – Screening Port (SP) has assembled a compound library of approximately 2,000 known drugs that are extensively annotated with respect to their primary targets. These compounds serve as a valuable tool to interrogate novel drug discovery targets. We have screened these compounds against a panel of 384-well microtiter plate based biochemical and cell-based drug discovery assays and suitable counter assays using our High Throughput Screening (HTS) system (Figure 1).[1-3]

**Figure 1:** the HTS system at the Fraunhofer for assay development and robotic screening.

The outputs of the various screening campaigns have been evaluated using industry standard analysis (ActivityBase XE) and visualization (Spotfire) software. We have identified chemical scaffolds which add confidence that the activity of the targets investigated can be modulated by small molecules and thus support the idea that they are druggable.[4] Examples of our work will be presented.

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## Poster communication 19 – WG 1

# Diversity-oriented syntheses of new sulfur-heterocycles as candidates for pharmaceutical applications

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The impact of sulfur-heterocyclic compounds in pharmaceutical industry is significant,[1] therefore the search for efficient diversity-oriented syntheses to provide this type of compounds represents a topical subject. Our research focuses on the development of new efficient atom and step-economical approaches to access original small and medium S-heterocycles, for therapeutic applications. Multicomponent reactions and metal-mediated domino processes, which represent powerful tools in the synthesis of various heterocyclic molecules, are particularly studied in our laboratory for this purpose.[2] In this communication will be presented three synthetic methods that we developed, giving access to valuable *S*- and *N,S*-heterocycles with a wide variety of structures (Figure 1): 1,3-thiazines, 2-amino-benzothiazoles, 5- and 6-membered thiacycles (i.e. benzothiolanes and isothiochromanes), and challenging medium-sized (> 7 atoms) thiazacyclic fused ring systems. Current efforts are focused on exploiting these scaffolds in medicinal chemistry.

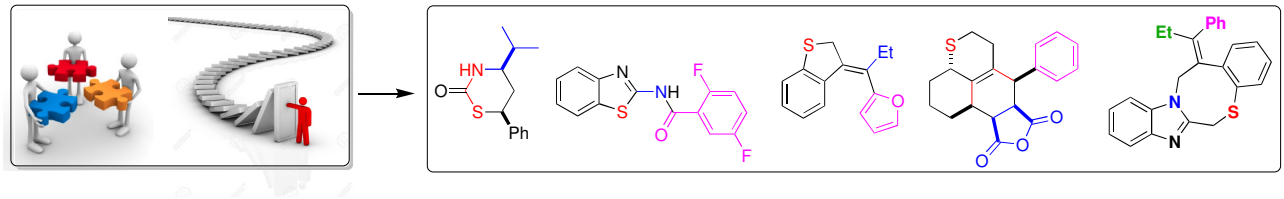


**Figure 1:** Examples of synthesized sulfur-containing heterocycles *via* multicomponent and domino reactions

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## Poster communication 20 – WG 1

# Multitarget Quinone-methide Triterpenoids from *Maytenus chiapiensis* as Potential anti-Cancer Agents

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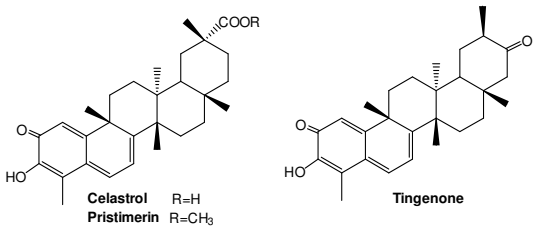
The *Celastraceae* family, commonly known as the bittersweet family, consists of 106 genera and 1300 species, distributed extensively in tropical and sub-tropical regions. The most representative genus is *Maytenus*, and its species are widely used in traditional medicine and agriculture in North Africa, South and Central America, and Central and East Asia. Indeed, bioactive celastroloids, pentacyclic triterpenes, sesquiterpene pyridine alkaloids, and dihydro-β-agarofuran sesquiterpenes have been reported from *Maytenus* species. Naturally occurring quinone-methide triterpenoids, chemotaxonomic markers of the *Celastraceae* family, are a type of celastroloid exhibiting a wide range of biological effects, including anti-inflammatory, antioxidant, antimalarial, insecticidal and antitumor activities. In particular, they have been reported to suppress the proliferation of breast, glioma, prostate, pancreatic, ovarian and colon human cancer cell lines.[1] In fact, the anticancer mechanisms of action of celastrol[2] and pristimerin[3] have been extensively reported. Moreover, these compounds cannot be obtained by chemical synthesis yet. Thus, their extraction from plants remains the only feasible strategy to obtain them, and biotechnological techniques, such as *in vitro* culture of cells, may become an alternative source in the future.

As a continuation of our research focussed on the discovery of novel anticancer drugs, the phytochemical study of *Maytenus chiapiensis*, an endemic plant from El Salvador, revealed this species is a rich source of promising anticancer quinone-methide triterpenoids. Herein on are reported the isolation of the quinone-methide triterpenoids, pristimerin and tingenone as the main components of the root bark extract of *M. chiapiensis*. The information reported here open the perspective to use these particular molecules as scaffolds for the design of novel and selective anticancer agents.

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## Poster communication 21 – WG 1

# Synthesis of 1,2,4-oxadiazoles and amidoxime-related derivatives as potential antimicrobial agents

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1,2,4-Oxadiazole ring has been found in bioactive natural products[1,2]. It was used as heterocyclic bioisoster of amide or ester in the synthesis of peptide building blocks and in the formation of dipeptidomimetics[3], that showed increased hydrolytic and metabolic stability[4]. In the field of medicinal chemistry, 1,2,4-oxadizole scaffold showed antimicrobial activity, e.g. antibacterial activity of a linezolid-based series[5] or in a group of non-beta-lactam penicillin-binding proteins inhibitors with 100% oral bioavailability[6]. There are several drugs containing the 1,2,4-oxadiazole ring, e.g. prenoxdiazin, pleconaril.

In this study, a series of *N*'-hydroxypyrazine-2-carboximidamides has been synthesized as starting material for synthesis of acylated derivatives, *N*'-hydroxypyrazine-2-carbimidoyl-chlorides and pyrazine-2-yl-1,2,4-oxadiazoles. All products characterized by means of melting points, IR and NMR spectra have been submitted for antifungal, antibacterial and antimycobacterial screening. Inhibitory activity against *Mycobacterium smegmatis* DSM 43465 (ATCC 607) and *M. aurum* DSM 43999 (ATCC 23366) has been detected. An *in silico* study on lipophilicity, conformation and configuration has been performed with *N*'-hydroxypyrazine-2-carboximidamides, *N*'-hydroxypyrazine-2-carbimidoyl-chlorides and pyrazine-2-yl-1,2,4-oxadiazoles. A comparison of calculated configuration and configuration determined with NOE experiments has been provided.

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## Poster communication 22 – WG 1

# Xanthone derivatives as histamine $H_3$ receptor ligands and cholinesterase inhibitors

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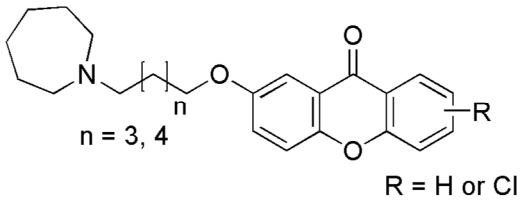
The histamine $H_3$ receptor ($H_3R$) is widely distributed in the brain, especially in the parts connected with memory and cognition. Its blockade increases the release of histamine itself as well as other neurotransmitters, such as acetylcholine, dopamine, noradrenaline or serotonin. Pharmacological studies suggested the utility of histamine $H_3R$ antagonists/inverse agonists in treatment of various human disorders, e.g. Alzheimer's Disease (AD), ADHD, Parkinson's Disease, schizophrenia, narcolepsy or allergy [1]. In the past years, in addition to selective $H_3R$ antagonists/inverse agonists multitarget (mostly dual acting) ligands were also obtained. Compounds were designed as structures exhibiting antagonism/inverse agonism at the $H_3R$ while simultaneously affecting other biological targets [2]. In this study, based on the general construction pattern of histamine $H_3R$ ligands [3], we designed xanthone derivatives and evaluated their potential inhibitory activity towards acetylcholinesterase (AChE) in a docking-based virtual screening. Then, 18 compounds were synthesized, evaluted for human histamine $H_3R$ affinity and cholinesterase inhibitory activity (AChE and butyrylcholinesterase) using the method established by Ellman et al. [4]. The target compounds showed human $H_3R$ affinities in nanomolar range ($K_i$ < 1000 nM) and exhibited cholinesterase inhibitory activity with $IC_{50}$ values in submicromolar range. Especially very promisning were azepane derivatives (**Figure 1**).




$hH_3R$ $K_i$ < 600 nM
AChE $IC_{50}$ < 350 nM
BuChE $IC_{50}$ < 1300 nM

**Figure 1**. General structure of azepane derivatives of xanthone.

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## Poster communication 23 – WG 2

# The predictive significance of Metastasis Associated in Colon Cancer-1 (MACC1) in primary breast cancer

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MACC1 (metastasis associated in colon cancer-1) is a prognostic biomarker for tumor progression, metastasis and survival of a variety of solid cancers. MACC1 also causes tumor growth in xenograft models and acts as a master regulator of the HGF/MET signaling pathway. In breast cancer, the expression of MACC1 determined by immunohistochemistry was significantly associated with positive lymph node status and advanced clinical stage. The aim of the present study was to further investigate the prognostic or predictive value of MACC1 expression in breast cancer using western blot analysis and immunohistochemistry. The results of our study have shown that high MACC1 expression in breast cancer is associated with shorter disease-free survival, especially in node-negative tumors. The MACC1 might be a suitable biomarker to select patients with a higher probability of recurrence which might benefit from adjuvant chemotherapy. Our results support a biologic role and potentially open the perspective for the use of MACC1 as predictive biomarker for treatment decision in breast cancer patients.

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## Poster communication 24 – WG 1

# Sustainable tandem process and chirality transfer in the synthesis of six and seven membered ring oxacycles

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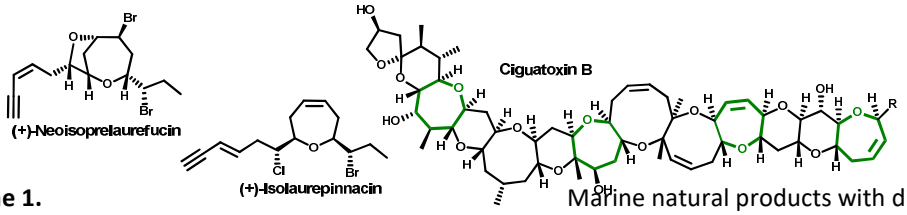
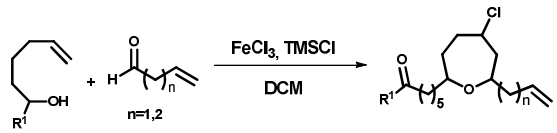
In recent years, iron has been one of most used metal catalysts due to low cost, environmentally-safe behavior and high catalytic activity. This activity is based on its strong Lewis acidity, very suitable in reactions such as Prins cyclization. In our group, we have studied different modifications of Prins cyclizations, using different Iron(II) and Iron(III) salts, to lead to six and seven membered oxacycles.[1] This type of oxacycles appears in many different marine natural products from small molecules such as isolaurepinnacin to bigger molecules such as ciguatoxin, brevetoxin, etc.




**Scheme 1.** Marine natural products with different type of oxacycles.

In this work, a new chirality transfer 1,5 hydride shift-Prins cyclization tandem reaction was studied under sustainable metal catalysis.




**Scheme 2.** Chirality transfer 1,5 hydride shift-Prins cyclization tandem reaction

This reaction combines two effective direct processes. First, we perform a Prins reaction with a 1,5 hydride shift and chirality transfer. In situ, the resulting hydroxylketone is involved in a additional Prins cyclization that leads the corresponding oxacycles. This family of compounds, with a few lateral chain modifications, can provide interesting structural motifs for biological assays.

Acknowledgments: We thank the Spanish MINECO, CTQ2014-56362-C2-1-P and ACIISI (Gobierno de Canarias) ProID2017010118 co-financed by the European Regional Development Fund (ERDF). JMLS thanks ACIISI-Gobierno de Canarias for a predoctoral fellowship

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## Poster communication 25 – WG 1

# Photo-Fenton reaction interfering ABTS peroxidase activity test for *salen*-manganese complexes

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*Salen*-manganese complexes are ROS scavengers whose catalytic and pharmacological properties have been studied for over 20 years.[1] In these studies, the *salen*-type ligands include other organic derivatives known as *salpn* or *salophen*, with different spacers between the aromatic rings. Our intention to deepen the mechanistic study of the peroxidase-like activity of this type of complex led us to realize how photo-Fenton processes can induce errors in the ABTS peroxidase test. In order to demonstrate this issue, we have chosen three artificial mimics **1**-**3** (Scheme 1) and also a well-known $H_2O_2$ scavenger such as the EUK-134 complex. The stability of these compounds against hydrogen peroxide when we use a filter to cut light of wavelengths shorter than 425 nm show the need to reevaluate their mechanism of antioxidant activity in the pharmacological applications in which they are currently used.

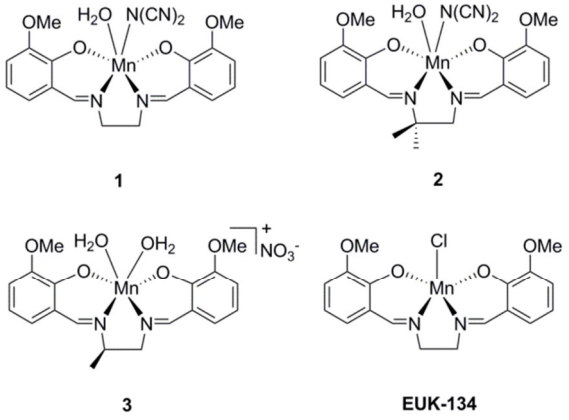


**Figure 1:** Structure of complexes **1**-**3** and **EUK-134**.

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## Poster communication 26 – WG 1

# A new family of densely functionalized fused-benzoquinones as potent human protein kinase CK2 inhibitors

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A new series of 2-amino-4-phenyl-6-hydroxy-7-alkyl-pyranobenzoquinones was synthesized as ATP-competitive CK2 inhibitors. They were readily synthesized through a three-component Knoevenagel condensation-Michael addition-heterocyclization reaction from aldehydes, malononitrile, and 3-alkyl-2,5-dihydroxybenzoquinones. Some of the synthesized compounds presented interesting inhibitory activity with $IC_{50}$ values in the submicromolar range. A structure-activity relationship study was carried out and the mode of binding was analysed by docking studies and supported by ATP competition assays.

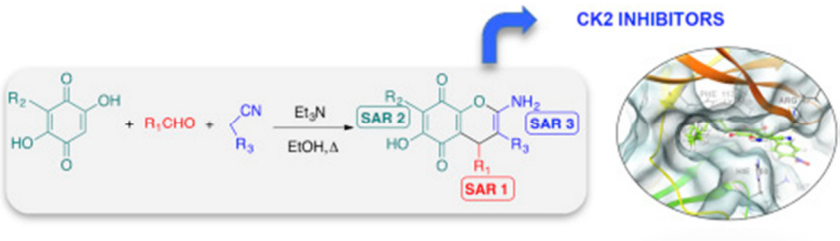


**Figure 1**

**Acknowledgments:** We thank MINECO (SAF2015-65113-C2-1-R) for financial support. P.M.A thanks ACIISI-EU for a predoctoral grant.

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## Poster communication 27 – WG 4

# *In silico* hit-identification of potential multi-targeting bioactive compounds extracted from essential oils endowed with kinase inhibition activity

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Designing drugs that can simultaneously interact with multiple targets is a promising approach for treating complicated diseases such as cancer. Compared to using combinations of single target drugs, multi-target drugs have advantages of higher efficacy, improved safety profile, and simpler administration. Many *in silico* methods have been developed to approach different aspects of this polypharmacology-guided drug design, particularly for drug repurposing and multi-target drug design.[1] Traditionally, Essential oils (EOs) have been used for their biological activities including antibacterial, antifungal, sedative, antioxidant, spasmolytic, carminative, hepatoprotective, and analgesic activities.[2] In a previous study, the EOs have been considered as possible resources of carbonic anhydrase inhibitors for the obesity treatment.[4] In silico structure-based virtual screening and in vitro assays showed EOs components as promising antiobesity agents. Actually, they are popular in aromatherapy for its curative effects. In this study, we have considered EOs as possible resources of new kinases inhibitors with a polypharmacological profile. With this aim, we have performed a SBVS by using a ligand library, downloaded from Essential Oil University website (EOU),[3] and X-ray models of several protein kinases selected from Protein Data Bank (PDB) website. Binding energies were evaluated and 13 compounds were detected as new potential hit compounds with a multi-targeting profile.

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## Poster communication 28 – WG 2

# Halogenated phenothiazines still underexplored as multitargeting agents

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Over the decades, phenothiazines have been exploited in many pharmacological areas; indeed, besides their well-known antipsychotic activity, also antiprotozoal, antifungal, anticancer, and antiprion activities have been described.[1] Phenothiazine derivatives have also been reported as antivirals for their ability to inhibit the Tat-mediated transcription of HIV-1, through the interaction with the nucleic acid TAR RNA.[2] Moreover, phenothiazines have been shown to exhibit anti *Mycobacterium tuberculosis* (Mtb) activity, also against multidrug-resistant Mtb, through the inhibition of multiple biological targets, such as NADH II dehydrogenase (NDH-2),[3] efflux pumps,[4] calcium-calmodulin binding,[5] and bacterial RNase P RNAs.[6]

In the search for potent and selective TAR ligands endowed with the ability to inhibit the HIV-1 Tat-mediated transcription, we initially designed and synthesized a first series of halogenated phenothiazines (HPs) with the aim to exploit the halogen bonding to improve the target recognition, an approach that have recently attracted the attention of medicinal chemists to design innovative compounds.[7] The lack of any anti-HIV-1 activity prompted us to repurpose these novel compounds for alternative applications.

In particular, considering the anti-Mtb activity reported for some therapeutic phenothiazines,[8] we decided to test the synthesized HPs against Mtb. In this case, very interesting results were obtained that permitted to delineate a preliminary structure-activity relationship and pave the way for a more in-depth investigation of the HPs as new anti-Mtb agents.

In this work, the design, synthesis, and biological evaluation of a series of HPs, will be presented.

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## Poster communication 29 – WG 2

# Electrochemical cysteamine assay for the study of reactivity of bioactive ligands

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The detection principle of the assay is based on electrochemical monitoring of cysteamine as model molecule (molecular probe) containing two redox centers, *i.e.* sulfhydryl and amino groups (Fig. 1A). An amine group can create Schiff bases with various aldehydes, and a sulfhydryl group can be easily oxidized to a disulfide and is a good nucleophile. A typical reaction is Thiol-Michael addition [1].

Electrochemical analysis was utilized for observing the interactions of free amino and thiol groups. Therefore, the method could be applied for investigating the reactivity of a broad spectrum of binding ligands to both redox centers of cysteamine. Also, the stability of a battery of thiols and amines under different conditions can be investigated by this method.

Two ligands with well known biological activities and functional groups with different reactivities are involved in the pilot study. Sulforaphane is a natural isothiocyanate (Fig. 1B) and 4-hydroxynonenal is an unsaturated aldehyde produced by lipid peroxidation (Fig. 1C). The reaction products of both ligands with cysteamine were identified using LC-MS.

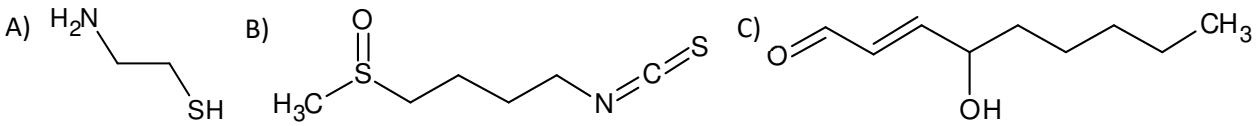


**Figure 1:** Structures of A) cysteamine, B) sulforaphane, C) 4-hydroxynonenal.

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## Poster communication 30 – WG 1

# Synthesis of antiplasmodial naphthoquinone-triazol conjugates

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Nowadays, malaria is still a main concern; the WHO estimates that 3 million people lives in areas where malaria transmission occurs[1] and the lack of efficacy of the current treatments makes necessary the development of new antimalarial agents.

1,4-Naphthoquinones are a class of compounds broadly studied in medicinal chemistry and natural products chemistry.[2] Within these studies, the antimalarial activity has been reported for several 1,4-naphthoquinones,[3] such as atovaquone, a hydroxynaphthoquinone derivative, that has been reported as an effective antimalarial drug against multiresistant parasites. On the other hand, triazoles have been shown to possess a number of desirable features in the field of medicinal chemistry. Guided by the concept of molecular hybridization, in which two or more different pharmacophoric units are covalently linked into a single hybrid molecule with superior affinity and efficacy as compared to the parent drugs[4], some triazole naphthoquinones have been prepared as trypanocidal agents. Thus, in this communication we report the synthesis of diverse 1,2,3-triazolyl naphthoquinone derivatives and their antimalarial activity.

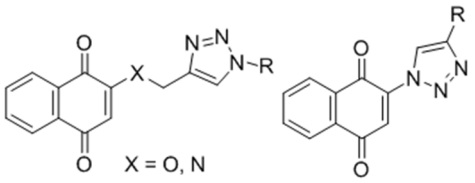


**Figure 1:** Synthesized 1,2,3-triazolyl naphthoquinone derivatives.

**Acknowledgements:** We thank MINECO (SAF2015-65113-C2-1-R) for financial support. P.L.R. thanks MECD for a predoctoral grant (FPU15/04896)

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## Poster communication 31 – WG 2

# Enhancing the bioavailability properties of a potent monoamine oxidase-B inhibitor using a nanotechnological approach

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of structure or function of neurons. Although many efforts have been made for the pursuit of successful therapies a great part of drug candidates fails in pre- and clinical trials due to several bioavailability setbacks. Recent advances in nanomedicine area have provided promising solutions for surpassing the point out constrains. Within this framework the use of polymeric nanoparticles (NPs) as drug delivery systems has been reported as an interesting tool to increase the stealth capacity of drugs to surpass biological barriers, while reducing low water-solubility issues.[1, 2]

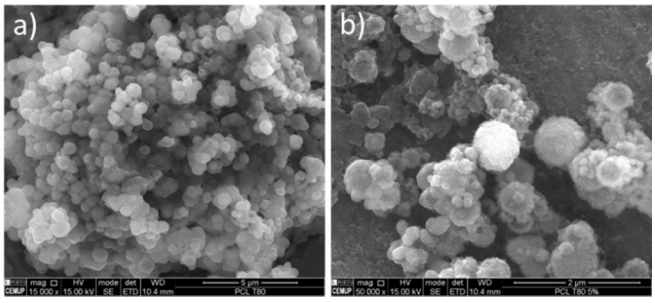


**Figure 1:** SEM images of a) unloaded and b) C27 loaded PEGylated coated PCL NPs.

Therefore, the aim of this work was the encapsulation of a novel chromone-based MAO-B inhibitor (C27) in PEGylated PCL NPs using the nanoprecipitation method.[3] C27 is a potent and reversible MAO-B inhibitor ($IC_{50}$ = 670 $\rho$M) inhibitor[4] that has some issues related with water solubility and BBB permeability properties that restricted its therapeutic application. The data related with physicochemical, morphological and bioavailability properties of the new nanoformulations will be presented in this communication.

**Acknowledgements:** This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145-FEDER-006980, and NORTE-01-0145-FEDER-000028). C Fernandes and J Reis grants were supported by FCT and FEDER/COMPETE funds.

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## Poster communication 32 – WG 1

# Memantine-isothiocianate: a new $H_2S$ releasing agent for neurodegenerative disease

Simona Sestito,[a] Simona Daniele,[a] Lorenza Bellusci,[b] Valentina Citi,[a] Vincenzo Calderone,[a] Grazia Chiellini,[b] Claudia Martini[a] and Simona Rapposelli[a]

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Alzheimer's disease (AD) is a progressive age-dependent neurodegenerative disease that has become a major public health issue with a significant impact on the whole society. Apart from acetylcholinesterase inhibition, Memantine, an uncompetitive, voltage-dependent NMDA receptor antagonist with moderate affinity and fast on-off kinetics, was the first novel agent for the treatment of AD and the only approved drug for moderate to severe AD therapy [1].

Since $H_2S$ has been recognized as a pleiotropic gasotransmitter with a role in neuroprotection and neuroinflammation and a potential value in AD therapy [2], we decided to replace the free amine group of the "native" drug Memantine with an isothiocyanate, i.e. a $H_2S$-releasing moiety (Figure 1).

The neuroprotective, antiradical and anti-Ab aggregation activities of this new chemical entity, namely Memit 1, were investigated. In addition, in light of the current investigations correlating neurodegeneration with an altered autophagic process, we also decided to explore the possible pro-autophagic effects. The results highlight that Memit 1 could represents an innovative multi-functional prodrug able to restore H2S levels and induce autophagy in the CNS, thus delaying the neurodegeneration processes and consequently, neurodegenerative disease progression.

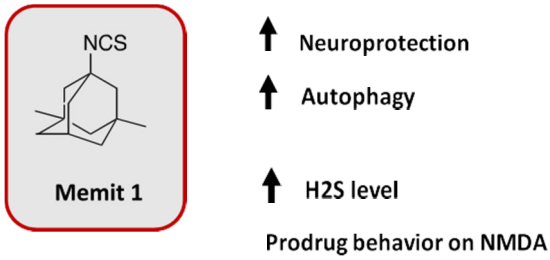


**Figure 1:** Memit 1.

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## Poster communication 33 – WG 1

# New method to synthesize medium and large size sultams

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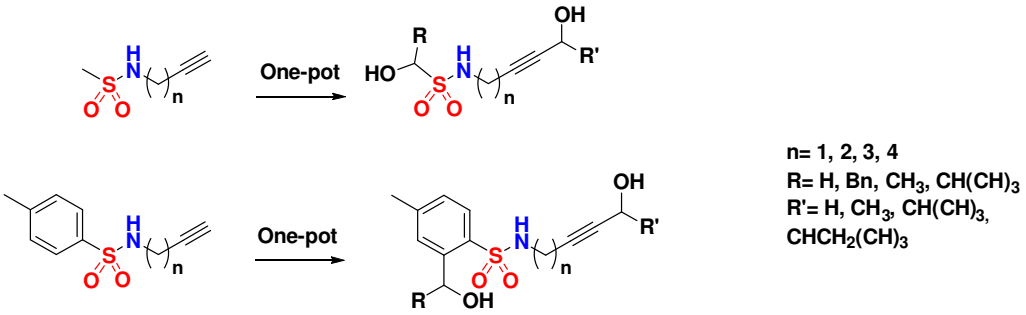
The growing demand for short synthesis of small molecules for high throughput screening has provided many opportunities and challenges for medicinal chemistry. A family of molecules with special interest for the pharmaceutical industry are the sultams (cyclic sulfonamides) due to their wide range of biological activities.1

Generally, the synthesis of sultams is based on the Friedel-Craft reaction, ring closing metathesis, cycloaddition [3 + 2], etc. Through these methods, sultams with rings between four and eight members are obtained.2 Therefore, new methods allowing the access to larger sultams are highly desirable.

Modifying *p*-toluenesulfonamide and methanesulfonamide groups, we have introduced structural complexity to molecules functionalizing groups usually employed as protecting groups. In addition, we could generate a set of molecules by selectively incorporating different aldehydes in a one-pot reaction. (Scheme 1) Through an intramolecular Nicholas reaction, these new molecules have afforded a collection of sultams with rings ranging from 10 to 13 members in few steps and good overall yields. (Figure 1).




*Scheme 1. Synthesis of sulfonamides using protective groups*

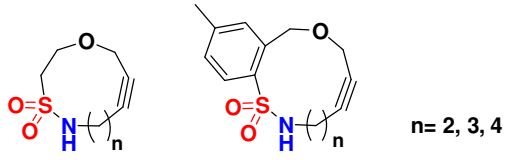



*Figure 1. Sultams of different sizes using protection groups*

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## Poster communication 34 – WG 1

# Stereoselective functionalization of $\Delta^2$-pyrrolin-4-ones

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Pyrrolones, i.e., hydroxypyrroles are privileged pharmacophores and are constituents of numerous natural and synthetic bioactive molecules (e. g. TDR32750 - anti malarial activity, Monopyrrolinone - HIV-1 protease inhibitor).

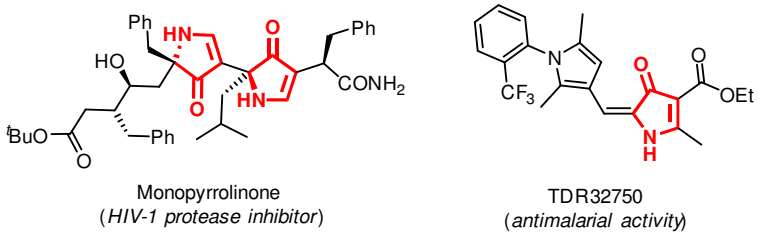


**Figure 1:** Examples of biologically active pyrrolones.

Asymmetric functionalization of enolizable heterocycles (i. e. pyrrolones) allows for an easy access towards non-racemic 3D-rich heterocycles, which are useful building blocks in the quest for new lead compounds. Even though pyrrolones have great applicative potential for diverse stereoselective transformations, they have seen very limited application in asymmetric electrophilic functionalizations. Easy availability of diverse 5-monosubstituted pyrrol-4-one nucleophiles from α-amino acids and the potential of non-racemic alkylated derivatives in the synthesis of complex pyrrolidine-based natural and synthetic products prompted us to study such transformations in detail. [1,2]

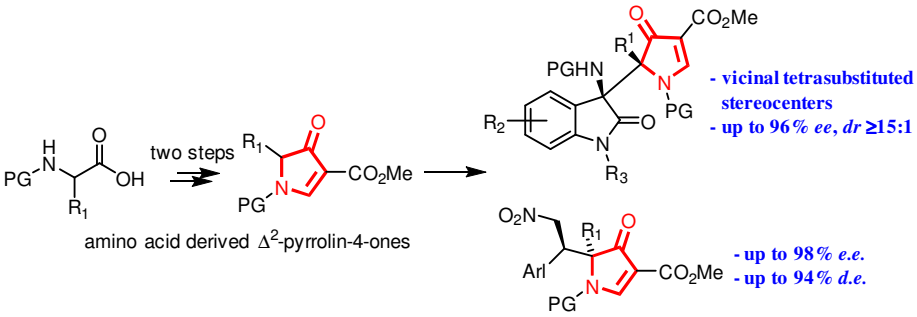


**Figure 2:** Representative stereoselective functionalizations of $\Delta^2$-pyrrolin-4-ones.

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## Poster communication 35 – WG 4

# Design of Novel Compounds with the Potential of Dual PPAR$\gamma/\alpha$ Modulation for the Management of Metabolic Syndrome

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This study sought to identify a single molecule capable of managing all three manifestations of metabolic syndrome-hyperglycaemia, dyslipidaemia and hypertension. Two Protein Data Bank (PDB) depositions were selected and used to establish the baseline affinity that any designed molecule in this study should ideally exceed in order to be considered for further optimisation. These were PDB depositions 3VN2 and 2P54 describing the bound co-ordinates of the Peroxisome Proliferator Activated Receptor (PPAR)$\delta$ partial agonist and Angiotensin II Receptor (Ang(II)R) blocker telmisartan and of the experimental PPAR$\beta$ fibrate agonist GW590735 bound to their respective cognate receptors. These small molecules were extracted from their cognate receptors, docked into their non-cognate counterparts, conformational analysis performed, and the optimal conformers were selected as template scaffolds in two parallel processes. The first was a fragment based *de novo* approach. Here, molecular moieties from the optimal telmisartan and GW590735 scaffolds modelled in their non-cognate targets and considered critical to binding were identified and modelled, in order to produce seed structures capable of sustaining molecular growth at user-directed sites designated as H.*spc* atoms subsequent to their being docked within the non-cognate Ligand Binding Pockets (LBPs). The second approach was a Virtual Screening (VS) exercise. Here, the optimal telmisartan and GW590735 conformers were submitted as query molecules to VS databases both individually and in the form of a consensus pharmacophore. This VS exercise identified structurally diverse molecules which were electronically and spatially similar to the queries and which were capable of modulating the target receptors. The molecular cohorts identified through both VS and the *de novo* approaches were filtered for Lipinski Rule compliance. The molecules that survived filtering were then re-docked into the non-cognate PPAR$\beta$ and/or $\delta$ _LBPs, conformational analysis re-performed and the affinity of the optimal conformer measured for its cognate receptor quantified. Comparison was made to the baseline and non-cognate receptor affinities previously established, and the molecules exhibiting dual affinities exceeding baseline values were selected for further optimisation. The use of the “tried and tested” Ang(II)R blocker and fibrate scaffolds as templates predisposes to the identification of novel structures devoid of unacceptable toxicity

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## Poster communication 36 – WG 1

# Synthesis of five-membered oxacycles through hydroxyalkoxylation

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Our research group has developed different methodologies to synthesize heterocycles of different size.[1,2] One of these are the five-membered oxacyles, which are present in many biologically active natural products. Recently, we have focused in the synthesis of these structures using the hydroalkoxylation reaction as key tool. It is a quickly and efficient methodology to achieve this type of important heterocycles in only two steps of reaction.

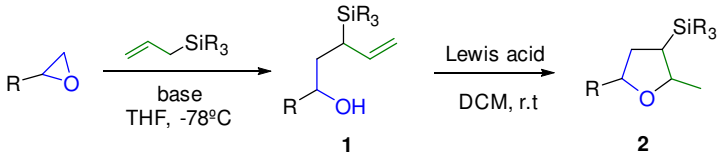


**Figure 1:** Direct synthesis of 2,3,5-trisubstituted tetrahydrofurans.

As **Figure 1** shows, we combine simple and efficient reactions to obtain 2,3,5-trisubstituted tetrahydrofurans. The opening epoxide reaction let the alcohol **1** with an allylic silyl group, which induce ring closure through its β-effect. Ring closure proceeds under mild conditions using a little amount of a Lewis acid.

In this communication, we will show the details of the research carried out to date as well as future ideas.

**Acknowledgments**: We thank the Spanish MINECO, CTQ2014-56362-C2-1-P and ACIISI (Gobierno de Canarias) ProID2017010118 co-financed by the European Regional Development Fund (ERDF).

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## Poster communication 37 – WG 1

# Antibacterial textile based on benzanthrone derivatives

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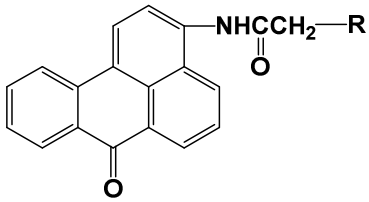
In the recent years the search for new effective bioactive agents with novel chemical structures has been a great concern worldwide and an impel of investigations in many countries. One of the good possibilities is to study the microbiological activity of organic compounds containing a system of conjugated double bonds which are prone to donor-acceptor interactions. Such compounds are used in textile chemistry as dyes for natural and synthetic fabrics. Antimicrobial wound dressings with incorporated biological active substances play an important role in prevention and management of wound infections. Their primary function is to provide a barrier between the wound and the environment. Thus the wound is protected from pathogens existing in the environment. On the other hand, antibacterial dressings also exert a broad spectrum of non-selective antibacterial action preventing the spread of infection. They act at multiple sites within the microbial cells, thus reducing the likelihood of bacteria to develop resistance. An interesting research area is combining the properties of substances with dyeing capacity with antibacterial and antifungal activity. That requires introduction of specific groups into the chromophores systems to achieve other antibacterial properties, and on the other hand, not to change their color performance.

Derivatives of benzo[*de*]anthracen-7-one (benzanthrone) are well known as fluorophores emitting fluorescence from yellow-green to orange-red. Their excellent color characteristics conditioned by the emitted fluorescence and high photo stability make them suitable for use in textile dyeing.




The synthesis and characterization of new benzanthrone derivatives and their deposition onto a cotton fabric are presented in this study. Their *in vitro* antimicrobial activity has been tested against Gram positive and Gram negative bacteria and yeast strains. The results obtained suggest that the newly synthesized compounds are effective in treating the relevant pathogens and are suitable for designing new effective antimicrobial preparations. Deposition of the benzanthrone derivatives on the textile fabric has been found to prevent the formation of a biofilm - a fact of significant importance in the production of antibacterial cotton fabrics. Hence, the new compounds have a high potential to be used for manufacturing healthcare and medical textiles.

**Acknowledgements**

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

## Poster communication 38 – WG 2

# The bile acids-based solutions for cell delivery of small molecule constructs

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We developed a fluorescence microscopy-based assay for the in-cell screening of bi(o)functional constructs. By utilizing this approach, we demonstrate that conjugation with bile acids overcomes a traditional problem faced in the design of non-zero-length linker-extended constructs – reduction in or no observed biological activity in the living cells.

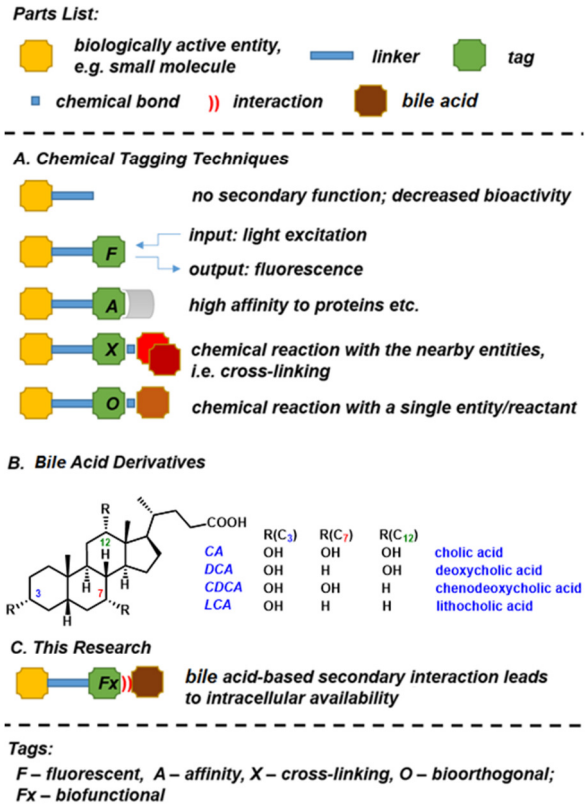


**Figure 1:** Bile acid-based modification of small molecules in the context of chemical labelling strategies.

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## Poster communication 39 – WG 1

# The interaction between oxyprenylated secondary metabolite and glucose transporter type 4

Vito Alessandro Taddeo[a], Salvatore Genovese[a], Serena Fiorito[a], Francesco Epifano[a], Isabel L. Bazzocchi[b]

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In recent years oxyprenylated secondary metabolites from plants, fungi, and bacteria and their semisynthetic derivatives have been a subject of growing interest. These natural products have been characterized as potential novel lead compounds for their wide range of pharmacological effects. In particular prenyloxy ferulic acid and umbelliferone derivatives including boropinic acid, isolated from the Australian shrub Boronia pinna Sm., 4'-geranyloxyferulic acid, 7-isopentenyloxycoumarin, and auraptene widespread in Citrus spp. were found to exert cancer chemopreventive, anti-inflammatory, anti-bacterial, and neuroprotective effects Glucose transporter 4 (GLUT4) is firmly established to play a pivotal role in glucose metabolism and in particular in modulating the insulin-stimulated glucose transport in several tissues, such as skeletal muscle and adipose tissue. Stimulation of GLUT4 by insulin results in its translocation to the plasma membrane, activation of several kinases, and a large glucose influx into cells. In this study we investigated the modulating properties of oxyprenylated ferulic acid and umbelliferone derivatives and their unprenylated parent compounds on GLUT-4 mediated glucose uptake and translocation.Methods: Oxyprenylated phenylpropanoids have been synthesized in high yields and purity by already reported methodologies. All the synthesized chemicals were tested for their capacity to modulate GLUT4 mediated glucose uptake and GLUT4 translocation in L6 rat skeletal myoblasts in the concentration range 0.1 – 10 µM. Insulin (0.1 µM) was used as positive control. Western blot analysis was employed to assess if GLUT4 translocation occurred prior to increase of glucose uptake. Statistical analyses were carried out by the Dunnett multiple comparison test. 4'-Geranyloxyferulic acid (GOFA), 7-isopentenyloxycoumarin, and auraptene (7-geranyloxycoumarin) increased glucose uptake in a concentration-dependent manner, and significant increases were observed at 0.1 µM for GOFA, and 10 µM for 7-isopentenyloxycoumarin, and auraptene. These products also were able to significantly promote the translocation of GLUT4 to the plasma membrane of L6 myotubes. After treatment with compounds for 15 min, the incorporated amounts of GOFA, 7-isopentenyloxucoumarin, and auraptene were 0.15, 0.32, and 1.77 nmols/60-mm culture dish, respectively. A sample of raw Italian propolis, found to be rich in GOFA and auraptene, mimic insulin-effect in the concentration range 0.01 – 1.0 mg/ml.

Conclusions: Among the compounds assayed, auraptene showed to be a potent activator of both translocation of GLUT4 and glucose influx into skeletal muscle cells with the highest bioavailability. Its capacity to modulate sugar metabolism, coupled to its presence in edible Citrus fruits, can be regarded as an additional reason to account for the already known stimulating properties of some vegetable (e.g. bitter orange).

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## Poster communication 40 – WG 2

# Mitochondria-targeted cinnamic antioxidants increase cellular stress response through modulation of mitochondria function/activity

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[a]*CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal;* [b]*CNC – Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech Building, Biocant Park – Cantanhede, Portugal.*

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Mitochondrial function and regulation of redox balance is fundamental in controlling cellular life and death pathways. Antioxidants have been used in order to counteract disruption of redox networks, normally associated with progressive loss of cell homeostasis and disease pathophysiology, although therapeutic success is limited mainly due to pharmacokinetic drawbacks. Attempts to improve mitochondrial function in several diseases spurred active drug discovery efforts and the development of new mitochondriotropic antioxidant agents, based on dietary polyphenols, has recently gained momentum. Phenolic acids such as hydroxycinnamic (HCA) are natural regulators of the cellular redox status and have pharmacological interest due to their intrinsic antioxidant properties.

Consequently, mitochondriotropic agents (AntiOxCINs) based on hydroxycinnamic acids were developed. AntiOxCINs toxicity was found to be dose-dependent and was only relevant for concentrations above those needed for their antioxidant activity. In fact, AntiOxCINs prevented iron- and hydrogen peroxide-induced cytotoxicity without disturbing mitochondrial function, morphology and polarization, and intracelular ATP. Moreover, AntiOxCINs altered the redox state of the treated cells and produced a mild increase in intracellular reactive oxygen species (ROS), which triggered an up-regulation of antioxidant defenses with no alterations on cell function and/or cell death. Instead, cellular GSH content and SOD levels were increased in cells treated with AntiOxCINs. Thus, it is likely that AntiOxCINs 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, due to their nature, mitochondria-targeted multi-functional antioxidants based on phenolic acids can stimulate stress responses and contribute to tissue protection, inhibiting directly or indirectly excessive mitochondrial ROS production. Thus, AntiOxCINs can be considered putative drug candidates to improve mitochondrial health in primary and/or secondary mitochondrial diseases.

**Acknowledgments.**

This work was funded by FEDER funds through the Operational Programme Competitiveness Factors - COMPETE and national funds by FCT - Foundation for Science and Technology under research grants PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440. J. Teixeira (NORTE-01-0145-FEDER-000028), R. Amorim (PTDC/DTP-FTO/2433/2014), F. Cagide (SFRH/BPD/74491/2010) and S. Benfeito (SFRH/BD/99189/2013) grants are supported by the European Regional Development Fund (ERDF) through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT–Fundação para a Ciência e a Tecnologia.

## Poster communication 41 – WG 1

# Discovery of potent dual DNA gyrase and topoisomerase IV inhibitors with broad spectrum antibacterial activity

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DNA gyrase and topoisomerase IV are validated targets for discovery of antibacterial drugs. These enzymes are heterodimers composed of two catalytic GyrA/ParC subunits and two GyrB/ParE subunits with ATPase activity. The latter have become attractive targets in many drug discovery projects in pharmaceutical industry, especially after successful introduction of the novobiocin into the therapy. However, novobiocin was later withdrawn from the market due to its unwanted side effects and development of bacterial resistance. Although many potent GyrB and ParE inhibitors with antibacterial activity, mainly against Gram positive bacteria, have been developed, none have yet reached the clinic.

Recently, we have discovered and optimized several structural classes of potent DNA gyrase and topoisomerase IV inhibitors possessing activity mainly against Gram positive pathogens.[1-3] Our latest optimization efforts resulted in the benzothiazole class of potent dual DNA gyrase and topoisomerase IV inhibitors possessing antibacterial activity against Gram positive (e.g. *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Enterococcus faecalis*) and Gram negative strains (e.g. *Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, Pseudomonas aeruginosa*). Furthermore, the best compounds display activity also against plasmid-mediated quinolone resistant *E. coli* strains, therefore, showing no cross-resistance with the fluoroquinolones. In addition, resistance potential in *E. coli* was determined and mutations were mapped to the residues in the ATPase domain of GyrB. Further in-depth studies are currently in progress to reveal true potential of the most advanced compounds as potential antibacterial agents.

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## Poster communication 42 – WG 1

# A new multitargeted drug candidate in alzheimer disease:

# 1,4-dihydropyridine-benzylidenhydrazon derived AChE inhibitor

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Several mechanisms are involved in pathogenesis of Alzheimer disease (AD). Development of novel acetylcholinesterase enzyme inhibitors (AChEI) and AChEI -based multi-target directed ligands (MTDLs) includes dual binding site. AChEIs and multi-target AChEIs inhibiting Aβ aggregation, regulating Aβ procession, antagonizing platelet-activating factor (PAF) receptor, scavenging oxygen radical, chelating metal ions, inhibiting monoamine oxidase B (MAO-B) or blocking N-methyl-D-aspartic acid (NMDA) receptors. Although cerebral vasodilators lowers AD symptoms, cerebral infarction increases AD incidence by 50% (2), the multitarget drug candidates targeting vascular protection is not studied enough. However considerable evidence from epidemiological, neuroimaging, pathological, pharmacotherapeutic, and clinical studies indicates that AD is a vascular disorder with neurodegenerative consequence (1-2). Previously we have shown that 1,4-dihydropiridine-benzylidenhidrazon derived C1 coded chemical that we synthesized inhibits AChE, Aβ fibril formation and causes destruction of already formed fibrils (IC50: 0.27 µM) (2). Because dihydropyridines are used in the treatment of hypertension, we tested whether C1 has vasodilatory effect or not to reveal multitarget drug potential of C1 in AD. We investigated the effect of C1 ($3.10^{-6}$-$10^{-5}$ M) on vascular tonus in mice aorta by DMT-wire-myograph. We have used $H_2S$ synthesis inhibitor PAG (10mM), NO synthesis inhibitor L-NNA (100uM) and Ca-free krebs buffer to evaluate the role of $H_2S$ and NO respectively on vasorelaxant effect of C1. We found that C1 ($3.10^{-6}$ M-$10^{-5}$M, n=8), causes relaxation in mice aorta precontracted with either phenylephrine of potassium chloride, suggesting that C1 causes relaxation which does not involve EDHF or potassium channels. C1-induced inhibition of phenylephrine-induced vascular tonus was significantly inhibited by PAG ($P<0.01$) or L-NNA ($P<0.001$). Beside calcium-induced contraction in the presence of Ca-free Krebs buffer is abolished by C1 ($10^{-5}$ M, 30 min. ($P<0.0001$). These result shows that C1 has vascular relaxant effect mainly through $Ca^{+2}$ channel blockage and partly through NO and $H_2S$. Thus multifunction of C1 on vascular protection, AChE inhibition as well as Aβ fibril inhibition may provide a new approach in treatment of AD as multitargeted drug candidate. **Acknowledgement**: We thank Turkish Scientific Research Council TUBITAK for the grant #114S448 and COST action CA15135.

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