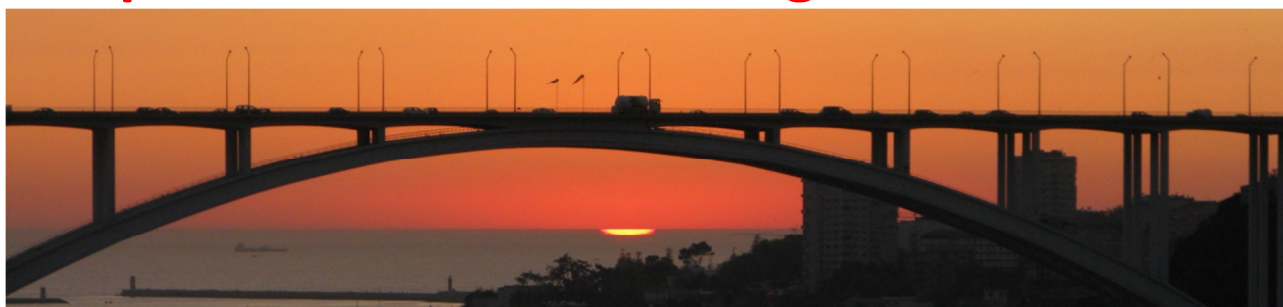


# Joint Annual Meeting 2017

## BOOK OF THE ABSTRACTS

EpiChemBio (CM1406) and MuTaLig COST (CA15135)  
actions joint annual meeting

## A bridge between EpiChemBio and MuTaLig COST Actions



Auditorium of the Portuguese Oncology Institute of Porto (IPO Porto)  
Rua Dr. António Bernardino de Almeida - 4200-072 Porto, Portugal  
22-24 September 2017



[www.epichembio.eu](http://www.epichembio.eu)



[www.mutalig.eu](http://www.mutalig.eu)



*EpiChemBio (CM1406) and MuTaLig (CA15135) COST actions joint meeting 2017 Porto (PT), Sept 22-24 2017*

## WELCOME

The Organizing Committee of the Joint Annual COST Action meeting, under the motto “A bridge between EpiChemBio and MuTaLig COST Actions”, will continue the tradition of previous COST meetings, hoping to contribute for fostering key topics on Epigenetic Chemical Biology & Multi-Target paradigm for innovative ligand identification in the drug discovery process. Besides, we also intend to offer to the participants a pleasant atmosphere in the beautiful city of Porto and the opportunity for the young researchers to interact closely with renowned experts and foster the exchange of ideas, experiences and networking with their peers.

You are also invited to visit Porto, with its historical centre (UNESCO World Heritage Site), the river Douro and the famous Port Wine cellars.

We are looking forward to meeting you in Porto and we hope this will be a fruitful and pleasant scientific event.

The Organizing Committee want to thank the Local Organizing Committee comprised by young investigators for their support

Anabela Borges  
Angela Marques Magalhães  
Daniel Chavarria  
Daniel Martins  
Fernando Cagide  
Inês Graça  
José Teixeira  
Maria Amorim  
Sofia Salta  
Tiago Silva

Special thanks goes to the young investigator Carmine Talarico (Università “Magna Græcia” di Catanzaro-Italy) and MuTaLig Chair for the invaluable assistance in the organization of the abstract book.



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## INTRODUCTION

Two COST Actions, working in overlapping areas of Medicinal Chemistry, decided in 2017 to organize a unique joint annual meeting. As only a few participants are members of both COST Actions EpiChemBio (CM1406) and MuTaLig (CA15135), the joint meeting will provide a forum for networking and discussion with mutual benefit. We hope this will be a successful new experiment with additional synergistic implications for everyone. The COST Association approved the proposal of the joint annual meeting in Porto from 22 to 24 September 2017.

Several criteria led to the choice of location, which was not by chance. Firstly, Portugal is within the COST list of ITC (Inclusiveness Target Countries) and the event will promote participation by local researchers. Secondly, Porto is an excellent choice, holding the title of Best European Destination 2017. Finally, we have excellent local organizers in Fernanda Borges who is a member of both Actions with the addition of Alexandra Gaspar, Rita Guedes and Manuel Simões from MuTaLig and Carmen Jerónimo and Cristiana Costa Pereira from EpiChemBio.

We believe all ingredients are perfect for a successful joint meeting. The program consists of eight thematic sessions, two combined sessions and a poster session with the two Actions alternating in the schedule. In total, 8 plenary lectures, 50 short oral presentations and 41 poster communications were accepted by the scientific board. We are pleased that a significant proportion are coming from early career investigators. In addition to the scientific sessions, there will be MC meetings of the two COST Actions, a social program and a final round table discussion.

As the Action Chairs, we want to express our deep gratitude to the Grant Holder of the MuTaLig COST Action from the University of Porto (Fernanda Borges and Joana Maria Neves Moreira Abrantes). We are indebted to the local organizers for all their hard work in putting together a meeting of this magnitude and to the COST Association (Lucia Forzi, Science Officer and Svetlana Voinova, Administrative Officer of both COST Actions) for their full support in making this joint activity a successful reality.

We wish a fruitful and stimulating meeting to all participants!

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## SUPPORTING INSTITUTIONS AND COMPANIES



**Equipamentos de Laboratório  
& Serviços.**



## PROGRAM

**Friday September 22<sup>nd</sup> 2017**

8:15 Registration and poster setup

8:45 **Introduction to the Joint COST Action annual meeting**

**José LARANJA PONTES** – President of Portuguese Oncology Institute, Porto (Portugal)

**A Fernando SILVA** – Dean of Faculty of Sciences, University of Porto (Portugal)

**Arminda ALVES** – LEPABE – University of Porto (Portugal)

**Fernanda BORGES** – University of Porto (Portugal)

**Carmen JERONIMO** – Portuguese Oncology Institute, Porto (Portugal)

**A GANESAN** (CA1406 Chair) – University of East Anglia, Norwich (UK)

**Stefano ALCARO** (CA15135 Chair) – Università “Magna Græcia” di Catanzaro (Italy)

### Session I “EpiChemBio: DNA and epigenetics”

**Moderator: Maria Berdasco MENENDEZ (Spain)**

9:30 SCE1 **Non-B DNA structures, as G-quadruplex and R loops, interact with each other regulating basic DNA functions and genome instability in human cancer cells**

**Giovanni CAPRANICO** – University of Bologna (Italy)

9:45 SCE2 **Molecular tools for targeted covalent functionalization and light-induced activation of biopolymers**

**Saulius KLIMASAUSKAS** – University of Vilnius (Lithuania)

10:00 SCE3 **Direct and indirect targeting of DNA methylation in cancers**

**Marie LOPEZ** – CNRS, Toulouse (France)

10:15 SCE4 **Anti-Neoplastic Activity of Newly Synthesized DNMT inhibitors in Renal Tumors**

**Ines GRACA** – Portuguese Oncology Institute of Porto (Portugal)

10:30 SCE5 **Targeted DNA demethylation in human cells by 5-methylcytosine excision**

**Jara Teresa PARILLA-DOBLAS** – University of Cordoba (Spain)

10:45 SCE6 **Epigenetic and antitumor effects of platinum(IV)-octanoato conjugates**

**Viktor BRABEC** – Czech Academy of Sciences, Brno (Czech Republic)

11:00 SCE7 **The relation between famine exposure and schizophrenia explained by cross-chromosome regulation of DUSP22 promotor hypermethylation**

**Marco BOKS** – University of Utrecht (Netherlands)

11:15 *Coffee break and poster session*

### Session II “MuTaLig on CNS part A”

**Moderator: Fernanda BORGES (Portugal)**

11:45 PLM1 **Multi-target ligands: more effective and sustainable drugs for Alzheimer’s disease?**

**Maria Laura BOLOGNESI** – “Alma Mater” Università di Bologna (Italy)

12:15 PLM2 **Development of donecopride as a potential multi-target directed ligand for the treatment of Alzheimer’s**

**Christophe ROCHAIS** – CERMN, Université de Caen Normandie (France)

12:45 SCM1 **The role of the chromone scaffold in the development of novel multitarget agents in Alzheimer’s disease**

**Joana REIS** – University of Porto (Portugal)

13:00 *Lunch*

### Session III “EpiChemBio: Histone acylation and deacylation”

**Moderator: Philippe BERTRAND (France)**

14:00 SCE8 **Targeting protein lysine acetylation in inflammatory lung diseases**

**Frank DEKKER** – University of Groningen (Netherlands)

14:15 SCE9 **Challenges in targeting histone acetyltransferases – A KAT8 case story**

**Hannah WAPENAAR** – University of Groningen (Netherlands)

14:30 SCE10 **In silico identification of isoform-selective HDAC inhibitors**

**Kemal YELEKCI** – Kadir Has University, Istanbul (Turkey)

- 14:45 SCE11 **Revisiting non-ribosomal macrocyclic peptides: Discovery of class I HDAC inhibitors with pM affinities**  
**Christian OLSEN** – University of Copenhagen (Denmark)
- 15:00 SCE12 **Erasing the eraser: Treating parasitic disease with epigenetics**  
**Remy NAROZNY** – University of East Anglia, Norwich (UK)
- 15:15 SCE13 **Design and synthesis of novel Histone Deacetylase 6 zinc binding groups**  
**Leandro AVELAR** – University of Düsseldorf (Germany)
- 15:30 SCE14 **Double life of the multifunctional disordered TPPP/P25: physiological function**  
**Adél SZABÓ** – Hungarian Academy of Sciences, Budapest (Hungary)
- 15:45 *Coffee break and poster session*

**Session IV “MuTaLig on CNS part B”**  
**Moderator: Rona RAMSAY (UK)**

- 16:15 PLM3 **Pain and Depression. Can be treated together?**  
**Dariusz MATOSIUK** – Medical University, Lublin (Poland)
- 16:45 PLM4 **Histamine H3 receptor antagonists acting as monoamine oxidase a/b inhibitors**  
**Holger STARK** – Heinrich Heine University Düsseldorf (Germany)
- 17:15 SCM2 **Novel multi-target ligands of aminergic GPCRS as potential antipsychotics**  
**Agnieszka KACZOR** – University of Lublin (Poland)
- 17:30 *Social program (optional)*

**Saturday September 23<sup>rd</sup> 2017**

**Session V “MuTaLig on Cancer and other targets”**  
**Moderator: Danijel KIKELJ (Slovenia)**

- 8:45 PLM5 **Kinetic, structural and biochemical characterization of asparagine synthetase inhibitors**  
**Nigel RICHARDS** – University of Cardiff (UK)
- 9:15 SCM3 **From cholinesterase inhibitors to multifunctional anti-Alzheimer's agents with anti-aggregating properties**  
**Barbara MALAWSKA** – Jagiellonian University Collegium Medicum, Kraków (Poland)
- 9:30 SCM4 **Targeting A<sub>1</sub> or/and A<sub>2A</sub> adenosine receptors and MAO-B to treat neurodegenerative Diseases**  
**Katarzyna KIEĆ-KONONOWICZ** – Jagiellonian University, Kraków (Poland)
- 9:45 SCM5 **Crosstalk between PDK1 and aurora kinase A: development of small multi-target agents**  
**Simona SESTITO** – Università di Pisa (Italy)
- 10:00 SCM6 **Diindolylmethanes (DIMs) as novel anti-cancer agents targeting GPR84 and cannabinoid receptors**  
**Thanigaimalai PILLAIYAR**, University of Bonn (Germany)
- 10:15 SCM7 **ABAD (17β-HSD10) inhibitors and their effect on key mitochondrial enzymes in the research of neurodegenerative diseases or cancer treatment**  
**Kamil MUSILEK** – University of Hradec Kralove (Czech Republic)
- 10:30 SCM8 **Development of an Antibody Radiolabeled Drug Conjugate (ARDC) using 195mPt-Carboplatin for Theranostic Approach in Ovarian Cancer**  
**Lisa-Maria REČNIK** – CNRS & Université de Montpellier (France)
- 10:45 SCM9 **Resveratrol analog induces hydrogen sulfide formation and vasorelaxation**  
**Gunay YETIK-ANACAK**, Ege University, Izmir (Turkey)
- 11:00 *Coffee break and poster session*

**Session VI “EpiChemBio: Histone Methylation”**  
**Moderator: A. GANESAN (UK)**

- 11:30 PLE1 **Regulatory elements and function of new lysine acylation pathways**  
**Yiming ZHAO** – University of Chicago, IL (USA)

- 12:00 SCE15 **From DNA methyltransferase transition state analogues to chemical scaffolds for the inhibition of PRMT4**  
Paola ARIMONDO – CNRS, Toulouse (France)
- 12:15 SCE16 **The quinazoline ring as a privileged scaffold in epigenetic (poly)pharmacology: from dual G9a methyltransferase/LSD1 demethylase inhibitors to selective LSD1 inhibitors**  
Dante ROTILI – University La Sapienza, Roma (Italy)
- 12:30 SCE17 **The Clinically Used Iron Chelator Deferasirox is an Inhibitor of Epigenetic JumoniC Domain-Containing Histone Demethylases**  
Martin ROATSCH – University of Leipzig (Germany)
- 12:45 SCE18 **Histone H3K27 Demethylase JMJD3 as a Therapeutic Target in Cancer**  
Patrick PERRIGUE – Polish Academy of Sciences, Poznan (Poland)
- 13:00 SCE19 **BLIMP1 and EZH2 in antibody secreting malignancies**  
Erna MAGNUSDOTTIR – University of Reykjavik (Iceland)
- 13:15 *Lunch*

Session VII “MuTaLig on in silico methods”

Moderator: Maurizio BOTTA (Italy)

EpiChemBio MC meeting in room 7

- 14:15 SCM10 **Updates about the Chemotheca tool: status of implementation and use**  
Stefano ALCARO – Università “Magna Græcia” di Catanzaro (Italy)  
Fernanda BORGES – University of Porto (Portugal)
- 14:30 PLM6 **Selective 3D-Pharmacophore models Targeted for Aldose Reductase: Medicinal Chemistry Decision Support and Activity Profiling**  
Sharon BRYANT – Inte:Ligand GmbH, Vienna (Austria)
- 15:00 SCM11 **Carbonic Anhydrase VA for the treatment of obesity: in silico identification of new inhibitors and prediction of anti-obesity side effects of FDA-approved drugs**  
Isabella ROMEO – Università “Magna Græcia” di Catanzaro (Italy)
- 15:15 SCM12 **Pharmacophore models for identification of DNA gyrase and topoisomerase IV inhibitors and evaluation of their off-target binding**  
Tihomir TOMAŠIČ – University of Ljubljana (Slovenia)
- 15:30 SCM13 **Identification of G-quadruplex DNA/RNA binders: structure-based virtual screening and biophysical characterization**  
Carmine TALARICO – Università “Magna Græcia” di Catanzaro (Italy)
- 15:45 SCM14 **BitterPredict- A Tool for Predicting Bitterness of Drug candidate from its Chemical Structure**  
Ayana DAGAN WIENER – The Hebrew University of Jerusalem (Israel)
- 16:00 *Coffee break and poster session*

Session VIII “EpiChemBio: Programming and Reprogramming”

Moderator: Carmen JERONIMO (Portugal)

MuTaLig MC meeting in room 7

- 16:30 SCE20 **Induction of Antitumor and Antifungal agents by Epigenetic Modifiers in the endophytic fungal strain *Dimorphosporicola tragani***  
Jose Ruben TORMO – MEDINA Foundation, Granada (Spain)
- 16:45 SCE21 **Meta-analysis of commonly deregulated miRNAs in oral cancer**  
Katarina ZELJIC – University of Belgrade (Serbia)
- 17:00 SCE22 **RESPONSE project: Chromatin regulators as biomarkers and combinatorial drug targets in colorectal cancer therapy**  
Sonia FORCALES – Germans Trias i Pujol Research Institute, Badalona (Spain)
- 17:15 SCE23 **Studying the Epigenetic Mechanisms and Biomarkers in Neuropsychiatric Diseases**  
Roberto Carlos AGIS-BALBOA – Galicia Sur Health Research Institute – IISGS, Vigo (Spain)

- 17:30 SCE24 **Environmental programming of respiratory allergy: utility of a child's spit epigenome**  
**Sabine LANGIE** – Flemish Institute for Technological Research, Mol (Belgium)
- 17:45 SCE25 **May epigenetics explain the serine proteases involvement in grapevine resistance to *Plasmopara viticola*?**  
**Joana FIGUEIREDO** – University of Lisbon (Portugal)
- 18:00 SCE26 **Epigenetic biomarkers for liquid biopsy–based testing of prostate cancer patients**  
**Sonata JARMALAITÉ** – Vilnius University (Lithuania)
- 18:30 *Port wine & cheese*

**Sunday September 24<sup>th</sup> 2017**

**Session IX “EpiChemBio & MuTaLig common topics”**

**Moderator: Paola ARIMONDO (France)**

- 9:00 SCC1 **Dual targeting with epigenetics**  
**A GANESAN** – University of East Anglia, Norwich (UK)
- 9:15 SCC2 **Cross metathesis for the synthesis of HDAC inhibitors. Potential in multitarget drug design**  
**Philippe BERTRAND** – University of Poitiers (France)
- 9:30 SCC3 **Photoactivatable platinum(IV) complex targeting genomic DNA and histone deacetylases**  
**Jana KASPARKOVA** – Czech Academy of Sciences, Brno (Czech Republic)
- 9:45 SCC4 **Novel fungal chitinase inhibitors and their possible use in Drug Discovery**  
**Maurizio BOTTA** – University of Siena (Italy)
- 10:00 SCC5 **Hybrid LSD1/JMJ Inhibitor As Therapeutic Option In Cancers With Dysregulated Hormone–Receptor Signalling**  
**Rosaria BENEDETTI** – University of Campania “Luigi Vanvitelli”, Napoli (Italy)
- 10:15 *Coffee break and poster session*

**Session X “EpiChemBio & MuTaLig common topics”**

**Moderator: Stefano ALCARO (Italy)**

- 10:45 PLC1 **Mitochondrial Toxicology in Drug Discovery and Development**  
**Paulo OLIVEIRA** – University of Coimbra (Portugal)
- 11:15 SCC6 **Naphthalene diimides and DNA: a variegated friendship**  
**Claudia SISSI** – Università di Padova (Italy)
- 11:30 SCC7 **Click chemistry a tool for medicinal chemistry**  
**Christian CAVÉ** – Université Paris Sud & CNRS (France)
- 11:45 SCC8 **Multi–level strategy for analysis of bioactive drug conformations**  
**Sanja ZIVANOVIC** – Institute for Research in Biomedicine, Barcelona (Spain)
- 12:00 SCC9 **Anticancer chalcones, selective or multitarget ligands?**  
**Bart ROMAN** – Ghent University (Belgium)
- 12:15 SCC10 **Smart screening libraries for academic use – General presentation – Building of an epigenetic library**  
**Marie Louise JUNG** – Prestwick Chemical, Strasbourg (France)

**Final Session: “EpiChemBio & MuTaLig round table and conclusions”**

**Moderators: Marianne ROTS (Netherlands) and Thierry LANGER (Austria)**

- 12:30  
**A GANESAN** (CA1406 Chair) – University of East Anglia, Norwich (UK)  
**Stefano ALCARO** (CA15135 Chair) – Università “Magna Græcia” di Catanzaro (Italy)  
**Fernanda BORGES** – University of Porto (Portugal)  
**Lucia FORZI** – Science Officer CA1406 & CA15135 COST Actions, Brussels (Belgium)  
**Marie Louise JUNG** – Prestwick Chemical, Strasbourg (France)



*EpiChemBio (CM1406) and MuTaLig (CA15135) COST actions joint meeting 2017 Porto (PT), Sept 22-24 2017*

## Plenary lectures

## Plenary Lecture Common Topics 1

### Mitochondrial Toxicology in Drug Discovery and Development

Paulo J. Oliveira<sup>a</sup>

<sup>a</sup> CNC – Center for Neurosciences and Cell Biology, University of Coimbra, UC-Biotech, Biocant Park, 3060-197 Cantanhede, Portugal

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Mitochondria are semi-autonomous organelles that play essential roles in cellular metabolism and programmed cell death pathways, as well as in the regulation of the redox environment. The several roles played by mitochondria in the context of cell metabolism, makes those organelles a critical player in the pathophysiology of different diseases, as well as a target for chemical toxicity<sup>1</sup>. In fact, mitochondrial toxicity has resulted in the withdrawal of several drugs from the market. One particular example is nefazodone, an anti-depressant withdrawn in the USA due to hepatotoxicity caused by drug-induced mitochondrial dysfunction<sup>2</sup>. Drug development and safety testing can involve the use of large numbers of laboratory animals, which, without a decisive pre-screening for mitochondrial toxicity, are often unable to pre-empt post-market entry fatal cases. The study of mitochondrial function in isolated fractions or cultured cells as a screening tool for increase drug safety can decrease the number of laboratory animals used in pre-clinical studies, thus improving animal welfare and healthcare outcomes and costs<sup>3-4</sup>. Novel techniques involving high-throughput methods can be used to investigate whether a drug candidate has associated mitochondrial toxicity. Moreover, these screens are mechanistically-based, since the effects of the drug on oxidative phosphorylation, calcium homeostasis and mitochondrial genetics can be assessed. This presentation is intended to demonstrate that mitochondrial toxicity studies are suitable for predicting drug and general chemical safety in toxicological screenings, critical in the process of drug development.

#### References

- <sup>1</sup> Pereira, S.P., Pereira, G.C., Moreno, A.J., Oliveira, P.J. Can drug safety be predicted and animal experiments reduced by using isolated mitochondrial fractions? *Altern lab Animal*, 2009, 37 (4), 355-365.
- <sup>2</sup> Silva, A.M., Barbosa, I.A., Seabra, C., Beltrão, N., Santos, R., Vega-Naredo, I., Oliveira, P.J., Cunha-Oliveira, T. Involvement of mitochondrial dysfunction in nefazodone-induced hepatotoxicity. *Food Chem Toxicol.* 2016, 94, 148-158.
- <sup>3</sup> Will, Y., Dykens, J. Mitochondrial toxicity assessment in industry--a decade of technology development and insight. *Expert Opin Drug Metab Toxicol.* 2014, 10(8), 1061-1067.
- <sup>4</sup> Pereira, C.V., Nadanaciva, S., Oliveira, P.J., Will, Y. The contribution of oxidative stress to drug-induced organ toxicity and its detection in vitro and in vivo. *Expert Opin Drug Metab Toxicol.* 2012, 8(2):219-37.

## Plenary Lecture EpiChemBio 1

### Regulatory elements and function of new lysine acylation pathways

Yingming Zhao

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We have recently identified eight types of new lysine acylation pathways: lysine malonylation, lysine succinylation, lysine glutarylation, lysine propionylation, lysine butyrylation, lysine crotonylation, lysine 3-hydroxybutyrylation, and lysine 2-hydroxyisobutyrylation. The new lysine acylation pathways are shown to have roles in epigenetic regulation and cellular metabolism. These pathways contribute to physiology changes and cellular dysfunctions associated with diverse inborn metabolic diseases. In this presentation, we will provide an overview of our understanding of new lysine acylation pathways. We will also report our recent progress on studying lysine beta-hydroxybutyrylation (Kbhb).

#### References

- Sabari, B.R., Zhang, D., Allis, C.D., **Zhao, Y.**, 2016. Metabolic regulation of gene expression through histone acylations. **Nat. Rev. Mol. Cell Biol.** **18**, 90-101.
- Xie, Z., Zhang, D., Chung, D., Tang, Z., Huang, H., Dai, L., Qi, S., Li, J., Colak, G., Chen, Y., Xia, C., Peng, C., Ruan, H., Kirkey, M., Wang, D., Jensen, L.M., Kwon, O.K., Lee, S., Pletcher, S.D., Tan, M., Lombard, D.B., White, K.P., Zhao, H., Li, J., Roeder, R.G., Yang, X., **Zhao, Y.**, 2016. Metabolic Regulation of Gene Expression by Histone Lysine  $\beta$ -Hydroxybutyrylation. **Mol. Cell** 62,194–206.



## Plenary Lecture MuTaLig 1

### Multi-target ligands: more effective and sustainable drugs for Alzheimer's disease?

Maria-Laura Bolognesi

<sup>a</sup> *Department of Pharmacy and Biotechnology, Via Belmeloro 6, Bologna, Italy*

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Alzheimer's disease (AD) represents one of the most formidable challenges for drug discovery. Although substantial investments and an ever-increasing clinical burden, an effective medicine has not been developed yet. As of 2016, there were an estimated 48 million people with dementia worldwide. This number will rise to 135.5 million in 2050, with much of the increase being in developing countries. Against this backdrop, multi-target drug discovery offers a concrete opportunity to develop medicines most suited to treating AD, i.e. safer, more effective, and affordable. Indeed, multi-target drugs opens up new prospects to tackle both efficacy and safety issues, which are key reasons for attrition and drug failure in AD.



In this respect, we have recently reported the first class of BACE-1/GSK-3 $\beta$  dual inhibitors based on a 3,4-dihydro-1,3,5-triazin-2(1H)-one skeleton, which displayed effective neuroprotective and neurogenic activities and no neurotoxicity in in vitro and in vivo assays.<sup>1</sup> We have also collected preliminary data supporting the idea that cashew nut shell liquid (CNSL) may play a significant role in the search for cost-effective and sustainable multi-target drugs.<sup>2</sup> Being a waste product of cashew nut food processing, it has great potential as a precursor for the production of high-value chemicals, especially drugs. On this basis and in connection with our studies on multi-target drugs, we have developed new chemical libraries of CNSL derivatives and investigated their therapeutic potential in AD.

#### References

- <sup>1</sup> Prati, F. *et al.* Multitarget drug discovery for Alzheimer's disease: triazinones as BACE-1 and GSK-3 $\beta$  inhibitors. *Angew. Chem Int. Ed. Engl.*, **2015**, 54, 1578-82; Prati, F. *et al.* 3,4-Dihydro-1,3,5-triazin-2(1H)-ones as the First Dual BACE-1/GSK-3 $\beta$  Fragment Hits against Alzheimer's Disease. *ACS Chem. Neurosci.*, **2015**, 6, 1665-82.
- <sup>2</sup> Lemes, L.F. *et al.* Cardanol-derived AChE inhibitors: Towards the development of dual binding derivatives for Alzheimer's disease. *Eur. J. Med. Chem.*, **2016**, 108, 687-700.

## Plenary Lecture MuTaLig 2

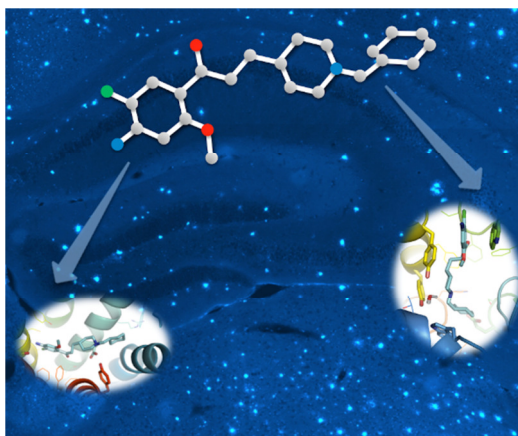
### Development of Donecopride as a potential Multi-Target directed ligand for the treatment of Alzheimer's

Rochais Christophe,<sup>a</sup> Dallemagne Patrick,<sup>a</sup>

<sup>a</sup> Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN) - UPRES EA 4258 - FR CNRS INC3M - SFICORE, Université de Caen Normandie, Faculté des Sciences Pharmaceutiques - Bd Becquerel, F-14032 Caen, France

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Targeting more than one molecular cause implied in the pathogenesis of Alzheimer's disease (AD) with a sole drug is considered a promising challenge, because it may address the numerous failures that recently occurred during clinical trials that were conducted in this area. The PLEIAD program has been launched few years ago in the CERMN with the objective to develop "Multi-Target-Directed Ligands" (MTDLs) with potential benefit to treat AD. We will present in this communication some of these recent examples and our own contribution to this field. Among the different candidate that we have identified we will more particularly discuss the case of Donecopride.<sup>1</sup>



**Figure 1:** X-Ray structure of Donecopride and docking with its targets

Donecopride is as a valuable dual (h)5-HT<sub>4</sub>R partial agonist ( $K_i = 10.4$  nM)/(h)AChEI ( $IC_{50} = 16$  nM) that further promotes sAPP $\alpha$  release ( $EC_{50} = 11.3$  nM). Donecopride could improve memory performances at 0.3 and 1 mg/kg on the object recognition test. In vivo results obtained in the Tg-5XFAD mouse model of AD will be for the first time presented. Based on these *in vitro* and *in vivo* activities, donecopride seems to be a promising drug candidate for AD treatment.

#### References

- <sup>2</sup> Lecoutey, C.; Hedou, D.; Freret, T.; Giannoni, P.; Gaven, F.; Since, M.; Bouet, V.; Ballandonne, C.; Corvaisier, S.; Malzert-Fréon, A.; Mignani, S.; Cresteil, T.; Boulouard, M.; Claeyen, S.; Rochais, C.; Dallemagne, P. Design of donecopride, a dual serotonin subtype 4 receptor agonist/acetylcholinesterase inhibitor with potential interest for Alzheimer's disease treatment *Proc. Natl.Acad. Sci. USA*, **2014**, *111*(36), E3825–E3830.
- <sup>3</sup> Rochais, C.; Lecoutey, C.; Gaven, F.; Giannoni, P.; Hamidouche, K.; Hedou, D.; Dubost, E.; Genest, G.; Yahiaoui, S.; Freret, T.; Bouet, V.; Dauphin, F.; Sopkova de Oliveira Santos, J.; Ballandonne, C.; Corvaisier, S.; Malzert-Fréon, A.; Legay, R.; Boulouard, M.; Claeyen, S.; Dallemagne, P. *J. Med. Chem.*, **2015**, *58* (7), 3172–3187.

## Plenary Lecture MuTaLig 3

### Pain and Depression. Can be treated together?

Matosiuk Dariusz, Bartuzi Damian, Kaczor Agnieszka A.

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For treatment of pain many different targets from CNS are elaborated. Same is for depression. Usually those targets are not common for both types of action although some drugs do exhibit elucidation of the depression syndromes associated with antinociceptive activity especially in high doses (e.g. benzodiazepines).

Very interesting combination of antidepressant and antinociceptive activity of new carbonyl derivatives of 1-aryl-2-aminoimidazoline is connected with their affinity to mu and delta opioid receptors [1-4]. It is proposed that their action could be dualistic on both receptors – allosteric in small doses with orthosteric affinity at elevated doses.



**Figure 1:** Allosteric (blue) and orthosteric (red) binding sites of MOP opioid receptors.

This way of action was confirmed by molecular modeling which included homology modeling of the proteins, docking of the ligands and validation of the models obtained by molecular dynamics of the formation of the water channel and PLS analysis [5,6]

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## Plenary Lecture MuTaLig 4

### Histamine H<sub>3</sub> Receptor Antagonists acting as Monoamine Oxidase A/B Inhibitors

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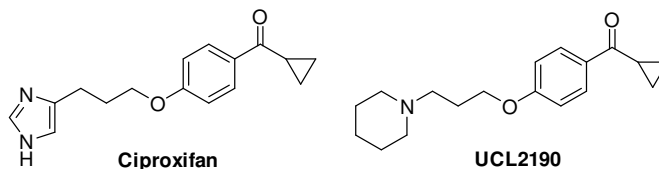
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The histamine H<sub>3</sub> receptor (H<sub>3</sub>R) as well as the neurotransmitter-catabolizing enzymes monoamine oxidase A and B (MAO A/B) are capable of neurotransmitter modulation, being highly investigated targets for therapy of neurological diseases, such as Parkinson's and Alzheimer's disease. Thus, the well-investigated, imidazole-containing, highly potent histamine H<sub>3</sub> receptor preferring antagonist/inverse agonist ciproxifan,<sup>1,2</sup> frequently used in rodent models for neurological diseases, and its non-imidazole analogue UCL2190 were evaluated for their MAO inhibition potential. In a screening for monoamine oxidase A and B inhibition properties ciproxifan showed inhibition of both enzyme isoforms. Further studies of ciproxifan revealed reversible inhibition with IC<sub>50</sub> values in the micromolar concentration range for both isoforms, slightly preferring MAO B in two species, human and rat.<sup>3</sup> Comparable IC<sub>50</sub> values for human MAO were also found for the analogous more potent human H<sub>3</sub>R antagonist UCL2190, showing even higher human MAO B preference compared to ciproxifan. Consequently, ciproxifan and UCL2190 might serve as promising starting leads for the development of multi-targeting H<sub>3</sub>R/MAO ligands, potentially suitable for treatment of neurological diseases.



**Figure 1:** H<sub>3</sub>R antagonists ciproxifan and UCL2190 with MAO inhibition capacities.

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## Plenary Lecture MuTaLig 5

### Kinetic, Structural and Biochemical Characterization of Asparagine Synthetase Inhibitors

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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. Metastatic sarcomas are often fatal, making the investigation of new therapeutic approaches desirable and timely, and we have recently shown that human asparagine synthetase (ASNS), the enzyme that synthesizes L-asparagine is elevated in sarcoma cells. Validating ASNS as a cancer target requires, however, access to potent cell-permeable, highly selective, small molecule ASNS inhibitors. In a breakthrough result, our efforts have led to the first success in obtaining a high-resolution X-ray crystal structure of human ASNS, and have modeled its interaction with an ASNS inhibitor that exhibits nanomolar potency. Access to this structural information, coupled with our ability to produce large quantities of human ASNS and a new synthetic procedure for obtaining unfunctionalized methylsulfoximines, will permit the development of new tools for use as chemical probes of sarcoma biology.

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## Plenary Lecture MuTaLig 6

### Selective 3D-Pharmacophore models Targeted for Aldose Reductase: Medicinal Chemistry Decision Support and Activity Profiling

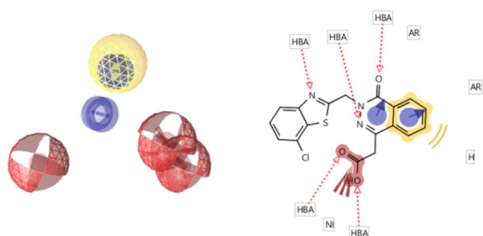
Sharon D. Bryant,<sup>a</sup> Bernabé Diéguez Roda,<sup>a</sup> Riccardo Martini,<sup>a</sup> and Magdalena Majeková<sup>b</sup>

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3D-pharmacophore modeling and virtual screening are techniques widely used by chemists [1] and modellers involved in pharmaceutical research to decipher key interaction features between proteins and ligands [2], find biologically active compounds [3], fish for new targets [4], repurpose existing drugs [5], explore protein-protein interfaces [6], and profile drug targets for side-effects [7]. During the last decade we have developed and expanded the capabilities of LigandScout, our molecular design platform, to further support medicinal chemists and modellers in their hit finding, hit expansion, hit to lead, and lead optimization research using advanced pharmacophore methodology.

In this study we developed selective 3D-pharmacophore models to identify novel inhibitors of aldose reductase (AR) as well as understand chemical features involved in selective inhibition of AR. AR is well known for its involvement in complications associated with diabetes mellitus. Although several AR inhibitors (ARIs) have progressed to clinical phases, all except epalrestat failed due to adverse side-effects. Nevertheless, recent research revealed that AR also mediates inflammatory signaling pathways involved in the development of different cardiovascular disorders and cancer. These findings have inspired interest in the search for new ARIs with novel scaffolds and improved side-effect profiles. The selective 3D-models developed in this study were used to filter and prioritize compounds with indole scaffolds targeted for AR with selectivity over aldehyde reductase [8]. Furthermore, the models are useful for in silico activity profiling to assess multi-target effects of hit compounds from virtual screens and to support lead optimization decision support to reduce side-effect liabilities.



**Figure 1.** A selective 3D-ligand-based pharmacophore model used for filtering compounds for activity at aldose reductase and selectivity over aldehyde reductase. The model was used to prioritize indole-based candidate compounds designed for AR.

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*EpiChemBio (CM1406) and MuTaLig (CA15135) COST actions joint meeting 2017 Porto (PT), Sept 22-24 2017*

## Short communications



## Short communication common topics 1

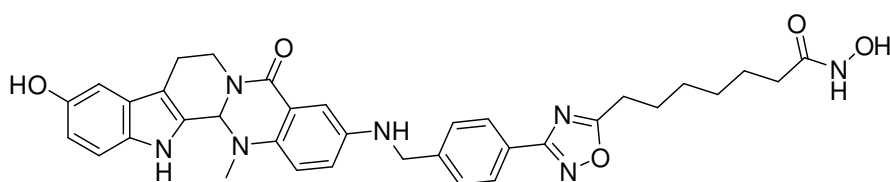
### Dual targeting with epigenetics

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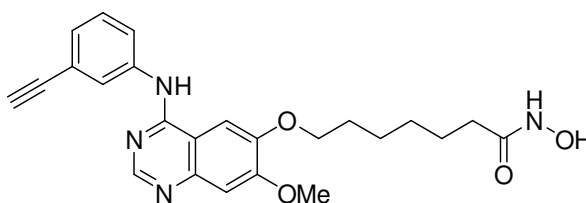
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The concept of dual targeting is increasing in popularity in epigenetics and there are many examples of hybrid drugs that combine an epigenetic mechanism of action with a non-epigenetic target. This review will cover successful strategies for compound design and dual target engagement from a medicinal chemistry point of view. Illustrative examples will include dual mechanism HDAC inhibitors from bench to bedside.



evodiamine analogue

*Triple inhibitor of topoisomerase I, topoisomerase II and histone deacetylases*



CUDC-101

*Nanomolar inhibitor of histone deacetylases, EGFR and Her in clinical trials*

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## Short communication common topics 2

### Cross metathesis for the synthesis of HDAC inhibitors. Potential in multitarget drug design.

Samuel Bouchet<sup>1</sup>, Camille Linot<sup>2</sup>, Dusan Ruzic<sup>3</sup>, Danica Agbaba<sup>3</sup>, Benoit Fouchaq<sup>4,5</sup>, Joëlle Roche<sup>5,6</sup>, Katarina Nikolic<sup>3</sup>, Muriel Cuendet<sup>6</sup>, Judit Ovádi<sup>7</sup>, Christophe Blanquart<sup>2,5</sup>, Philippe Bertrand<sup>1,5\*</sup>.

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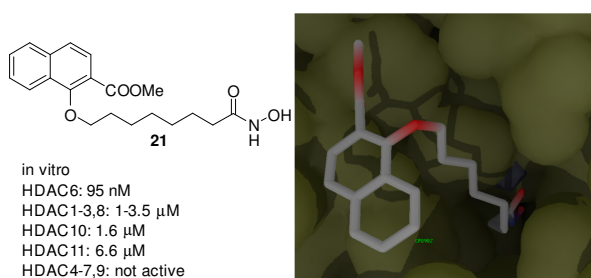
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Histone deacetylases<sup>1</sup> represent a family of eleven zinc-dependant enzymes. Their over expression has been correlated to several human diseases, in particular cancers. The search for compounds able to selectively inhibit one of these HDAC is of high importance to obtain less side effect during treatment as well as avoiding of target effects. In this work we have designed a series of inhibitors using an asymmetric cross metathesis approach. We present<sup>2,3</sup> the synthesis, some molecular modelling and the biological activities of the prepared compounds.



**Figure 1:** Identified selective HDAC6 inhibitor

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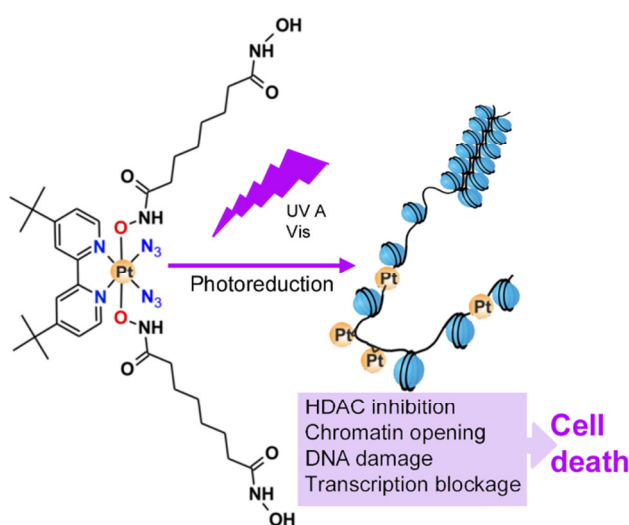
### Short communication common topics 3

## A photoactivatable Platinum(IV) complex targeting genomic DNA and Histone Deacetylases

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We designed a photoactivatable platinum(IV)-diazido complex, *cis,trans*-[Pt(N<sub>3</sub>)<sub>2</sub>(Sub)<sub>2</sub>(tBu<sub>2</sub>bpy)] (**1**), with suberoyl-bis-hydroxamic acid (SubH) as axial ligands, where tBu<sub>2</sub>bpy stands for 4,4'-di-tert-butyl-2,2'-bipyridine.<sup>1</sup> SubH is a histone deacetylase (HDAC) inhibitor, which inhibits the activity of HDACs at submicromolar concentrations and exhibits a profound dose-dependent inhibition of tumor cell proliferation at micromolar concentrations. Interestingly, SubH also shows potent synergistic interaction with antitumor platinum(II) complexes at equimolar concentrations. Thus, **1** was designed to achieve a dual functionality of the new prodrug, simultaneously targeting genomic DNA and HDACs both induced by low energy UVA or Vis irradiation. Our strategy has been to develop a new photoactivatable platinum(IV) system which is kinetically inert (in the dark)

to suppress the substitution reactions with biomolecules, but should be capable of controlled release by irradiation of clinically effective levels of platinum(II) species binding nuclear DNA as well as bioactive ligands (HDAC inhibitor) that may potentiate toxic effects of the platinum(II) drugs by an independent pathway. Notably, the substitutional inertness of the new system in the dark, as well as the relative stability of platinum(IV) complexes to hydrolysis, would prevent the premature release of biologically active platinum(II) and HDAC inhibitor species *in vitro* or *in vivo*. This approach relies on the use of a photoactivatable platinum(IV) pro-drug with a dual mechanism of action for localized tumors accessible by fiber-optic devices, thereby also decreasing the damage to normal, noncancerous cells.

### Acknowledgements

The authors acknowledge support from the Ministry of Education, Youth and Sports of the CR under Grant LTC17003. The authors also thank to prof. Travnicek and his coworkers for providing the platinum complex for this study.

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## **Short communication common topics 4**

### **Novel fungal chitinase inhibitors and their possible use in Drug Discovery**

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In the last ten years, we identified and developed a new therapeutic class of antifungal agents, the macrocyclic amidinouras. These compounds are active against several *Candida* species, including clinical isolates resistant to currently available antifungal drugs. We identified the Chitinase enzyme as target for these compounds thanks to an in-silico target fishing procedure, and biological evaluation highlights a strong inhibition of this enzyme. The significant activity they show against azole-resistant strains makes these compounds play a key role in the fight against antifungal resistance.

## Short communication common topics 5

### Hybrid LSD1/JMJ Inhibitor as therapeutic option in cancers with deregulated Hormone-Receptor Signalling

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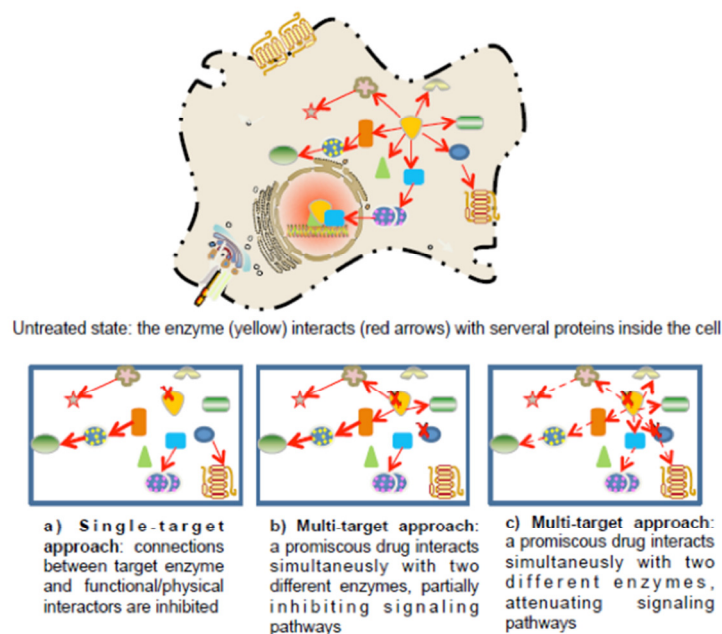
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The field of epigenetic-based drug discovery is currently in a transitional phase where the search for putative drugs is shifting from single-target-oriented molecules to network-active compounds (figure 1). The overall goal of the designed multiple ligand (DML) approach is to enhance the efficacy and/or improve the safety of therapy with respect to drug combination. In many human cancers, LSD1 and JmJc enzymes are co-expressed and co-localize with hormone receptor (AR, ER, PML-RAR), suggesting the potential use of hybrid molecules to target both enzymatic functions and to regulate/reindirect hormone receptor signalling. Here we report and characterize a novel “pan-KDM” inhibitor, obtained by coupling the chemical features of tranilcypromine, a known LSD1 inhibitor, with the 2OG competitive moiety developed for JmJc inhibition (**1**). The hybrid molecule displays unique features when compare with scaffolds and well-known single inhibitors. This compound efficiently inhibits LSD1 as well as JMJC enzymes in vitro, such as it induces high growth arrest and apoptosis in hormone responsive cells accompanied with a strong increases in levels of H3K4me2/3, H3K9me2/3 and H3K27me3. The treatment with hybrid molecule reduces the level of ER (in MCF7), AR (in LnCaP) and PML-RAR (in NB4), by both a transcriptional and non-transcriptional way. The same treatment changes the methylation status of ER/AR/PML-RAR regulated promoter regions, affecting the transcription of genes involved in cell surveillance, differentiation and epithelial-to-mesenchymal transition. Also in ex-vivo blasts and breast cancers, the hybrid molecule reduces cell proliferation, by interfering with hormone receptor signalling and/or receptors stability. In cellular models with acquired resistance to hormone stimuli (LnCaP C4-2), hybrid molecules is however able to inhibit cell proliferation, rebalancing the epigenetic signature of histones. In these systems the multiple-target inhibitor may overcome potential mechanism(s) of resistance caused in part by redundancy and robustness of biological pathways, founding an application field in the treatment of resistant to therapy cancers (triple negative breast cancers and cancers with acquired tamoxifen/enzalutamide/bicalutamide resistance).



**Figure 1:** Single-target versus multi-target approach. The cellular network, composed by several molecular entities (shapes) interacting with each other (arrows) in a physical or functional way, is schematically reported. Shapes and arrows are both potentially druggable. The node is here reported as a point connected by a large number of arrows. Arrows proceed from one molecule to another, and have different prevalence as indicated by thicker lines **(2)**.

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## **Short communication common topics 6**

### **Naphthalene diimides and DNA: a variegate friendship**

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DNA represents one of the most successful targets for anticancer therapies. It derives that since the approval of anthracyclines for clinical use, a huge number of small molecules designed to recognize the double helix have been synthesized, studied and, for some of them, the clinical efficiency was also confirmed. The main drawback related to the use of these drugs rests in severe side effects. The classic strategies to overcome this issue were based in synthetic effort devoted to increase the affinity for the DNA or to drive them to selected genomic portions.

Apparently, the so-obtained improvements are somehow limited but old molecules can still provide us unexpected novel perspectives.

Here we will use a well known pharmacophoric scaffold, naphthalene diimide, to show how when studied in simple models can lead to the design of molecules able to actually target DNA but simultaneously at different levels. Thus, this class of compounds represents an interesting example of multitarget-ligands which preferentially bind one single macromolecule but with multiple activities. The possibility to balance between these functions according to their structure will be discussed. In particular this aspect will be related to the DNA topological context.



## Short communication common topics 7

### Click chemistry a tool for Medicinal Chemistry

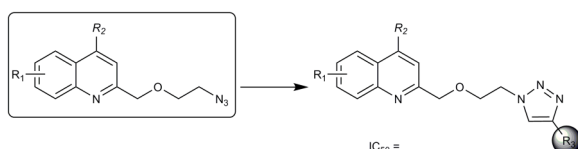
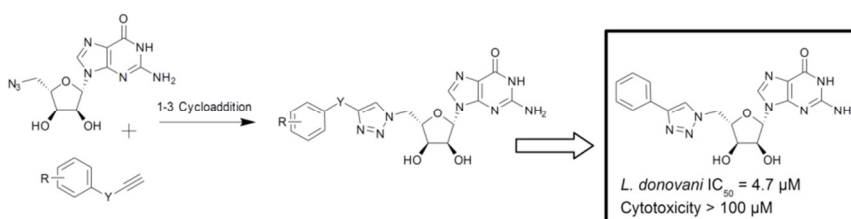
Christian Cavé,<sup>a</sup> Pierre Daligaux,<sup>a</sup> Sebastien Pomel,<sup>a</sup> Sandrine Cojean,<sup>a</sup> Philippe Loiseau<sup>a</sup>

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The development of chemical reactions has long held fascination in the field of medicinal Chemistry. The copper-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes has become a useful tool in medicinal chemistry to access a large diversity of structures exhibiting extensive biological activities [1]. This reaction is wide tolerant toward a large variety of functional groups, e.g., carboxylic acids, amines, alcohols, amides, and others, and is broadly known as the azide/alkyne-“click”-reaction or CuAAC-reaction [2]. Monocyclic 1,2,3-triazoles and benzotriazoles are remarkably stable towards hydrolysis, oxidative/reductive conditions, and enzymatic degradation.

Our laboratory is involved in the discovery and use of such reactions. Recent work by our team has identified new compounds with antileishmanial and/or antimalarial properties.



	<i>P.f. 3D7</i> IC <sub>50</sub> (μM)	<i>P.f. W2</i> IC <sub>50</sub> (μM)	R.I.	Cytotoxicity RAW264.7 CC <sub>50</sub> (μM)	S.I. = CC <sub>50</sub> /3D7 IC <sub>50</sub> <sup>a</sup>
<b>25f</b>	4.43 ± 0.08	1.56 ± 0.14	0.4	23.09 ± 7.46	>5.21
<b>25g</b>	1.85 ± 0.08	3.1 ± 0.26	1.7	56.32 ± 3.72	>30.44

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## Short communication common topics 8

### 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 Å 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. It is worth noting that present framework for multilevel strategy is a complex and long-term task, which requires a lot of rehearsals and implementations. 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.

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## Short communication common topics 9

### Anticancer chalcones, selective or multitarget ligands?

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We have been developing members of the chalcone chemical class as novel anti-invasive agents with potential for the study and treatment of metastatic cancer [1]. Some of the prototype molecules also exert cytotoxic effects against immature leukemia cell lines [2]. In both cases, the compounds show a sharp structure-activity relationship and act selectively against a subset of cell lines. The molecular targets of these molecules are unknown, but we have been able to differentiate them from known mechanisms involved in invasion and metastasis. We have also shown that these chalcones do not bind molecular targets commonly associated with chalcones.

In the present communication we will discuss these results, and comment on a number of forthcoming questions related to polypharmacology, multitarget activity and PAINS (pan-assay interference compounds) behavior, in the positive and negative sense. Are chalcones drugable? Do they display PAINS behavior? How can we explain our observed selectivity against a body of chalcone literature claiming affinity for a multitude of targets [3]? How are we planning to identify the molecular targets of our molecules? Finally, we will also present some opportunities for their development into multitarget ligands.

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## **Short communication common topics 10**

### **Smart screening libraries for academic use – General presentation**

#### **Building of an epigenetic library**

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Prestwick Chemical, created 1999, has a worldwide recognition for providing smart screening libraries, whereof the Prestwick Chemical Library, made of drugs out of patent is the flagship. Examples of repositioned drugs into various new applications will be presented. Other libraries, including a Fragment collection, are reported for their high interest in academic labs & institutions worldwide.

Moreover, building of an epigenetic library based on methyltransferase inhibition is currently ongoing and the first outcome will be detailed.

Prestwick has as well an impressive track record of compounds designed and prepared at Prestwick reaching clinical phase can be claimed: currently 10 molecules are developed in the clinic (phase I to phase III), coming out of our lead optimization work, and one is on the market in oncology.

## **Short communication EpiChemBio 1**

### **Non-B DNA structures, as G-quadruplex and R loops, interact with each other regulating basic DNA functions and genome instability in human cancer cells.**

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G-Quadruplexes (G4s) and R-loops are non-B DNA structures that can regulate transcription and replication. G4s are formed from four guanine residues that are held together in the same plane by Hoogsteen hydrogen bonds and further stabilized by the presence of monovalent cations. R-loops are triple-strand structures that contain an RNA-DNA hybrid and a displaced single-stranded DNA. R-loops and G4s are generally regarded as highly deleterious, indeed the structures can block both transcription and DNA replication, creating replicative stress and potentially causing DNA damage. Two of the most important features that influence these structures are the DNA torsional tension and the GC content. R-loop structures can be favoured by negative supercoils and a high guanine density in the non-template DNA strand. The latter is due to a higher thermodynamic stability of hybrids duplex. However, it remains to be shown directly whether Top1 activity may modulate R loop structures at specific genomic loci in human cells. On the other hand, it is not clear whether the two structures can interact in nuclear chromatin of living cells. Thus, here I will report our finding on two related projects.

In the first one, we performed DRIPc (DNA-RNA immunoprecipitation) analyses coupled to NGS to obtain high-resolution strand-specific genomic maps of R loops following Top1 depletion in human HEK cells. The results showed that Top1 downregulation induces an overall increase of R loops that are mainly localized in the body of genes. Interestingly, long genes are specifically affected by Top1 depletion, with an increase in R loop that was proportional to gene length. RNA-seq revealed that these genes tend to be highly expressed compare to genes on average. Surprisingly, a significant number of genomic loci showed a reduction of R loop peaks, mainly localized at ene-rich regions overlapping H3K27me3-marked active replication origins. Interestingly, Top1 depletion coincided with a block of the cell cycle in G0/G1 phase and replication delay. Our findings reveal new properties of Top1 in regulating R-loop homeostasis and suggest a potential role for Top1 in controlling replication origin via R-loop formation. The data directly show that Top1 can modulate R loop structures at specific loci according to the genomic context and provide new insights into Top1-dependent mechanisms of transcription regulation and genome stability.

In the second project, we have used immunofluorescence analysis in order to identify the increase of G4s and R-loops in cancer cells treated for 24 hours with different G4 binders. We have detected an R loop increase that is in parallel with G4 formation induced by all the tested G4 binders whereas inactive analogs do not stimulated R loop formation. The induction of R loops by G4 binders will be presented in relation to the cell killing activity of the tested compounds and to DNA cleavage and genome instability triggered by them.

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## Short communication EpiChemBio 2

### Molecular tools for targeted covalent functionalization and light-induced activation of biopolymers

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Functional analysis of biological systems requires the ability to visualize and manipulate selected biomolecules in their complex environments, which is often a challenging task. We have developed two novel platforms for targeted covalent manipulation of biopolymers. The first approach relies on methyltransferase enzymes, which catalyze highly specific methyl group transfers from the ubiquitous cofactor S-adenosyl-L-methionine (AdoMet) to a multitude of biological targets in the cell. We redesigned the methyltransferase reaction for covalent transfer of larger chemical entities by engineering the catalytic center and employing synthetic AdoMet analogs with transferable reporter or functional groups<sup>1,2</sup>. The new approach provides a new enabling tool for a highly targeted functionalization and labeling of natural DNA and tRNA, miRNA, rRNA<sup>3-6</sup>. We have also developed a universal technique for incorporation of a genetically-encoded photocaged selenocysteine residue into any position of a recombinant protein. Selenocysteine is of significant technological importance as a component of both natural proteins and designed biocatalysts, however the availability of such proteins is hampered by technical limitations. We demonstrate that photodecaging of the incorporated residue unmasks a unique chemical functionality (selenol), which may serve as an efficient site for light-induced protein dimerization inside yeast cells or permit site-specific labeling of the recombinant protein<sup>8</sup>. Altogether, the proposed approaches offer unprecedented flexibility in chemical manipulation of biomolecules for cellular imaging, chemical probing of biological interactions and spatiotemporal control of protein function.

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## Short communication EpiChemBio 3

### Direct and indirect targeting of Dna Methylation in cancers

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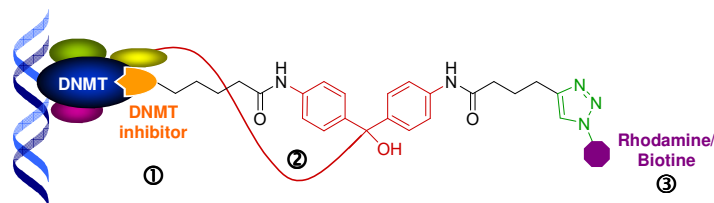
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DNA methylation is an epigenetic modification catalyzed by DNA methyltransferases (DNMTs). In cancers, hypermethylation of tumor suppressor gene (TSG) promoters is associated with their silencing, which participates to tumor formation, maintenance and progression. Only two nucleosides analogues, responsible for severe side effects, are today used as treatment. Our team researches aim at targeting DNA methylation in cancer cells to restore a normal methylation profile and slower tumor progression.

First, starting from screening results, we carried out optimization of a DNMT inhibitor (DNMTi) family and identified bromo-nitro-flavanone as potent DNMTi. We showed that some compounds have a micromolar activity on the purified enzyme and exhibit a more potent activity in our cellular model compared to initial compound. Additionally, their stability in physiological media was greatly improved.

Secondly, we applied a chemical-biology strategy to identify DNMT partners responsible for the guiding of DNMTs to the TSG promoter regions in cancers using the activity-based protein profiling (ABPP) method directly in living cells. From previously identified DNMTi, we designed and synthesized chemical tools containing: 1) a DNMT inhibitor entering in the active site of the DNMT, 2) a UV-crosslinking agent to trap proteins upon irradiation, and 3) an alkyne to be tagged by a fluorescent or affinity moiety.



**Figure 1:** ABPP applied to the identification of DNMT partners in cancers cells.

Evaluation of the anti-DNMT activities of these chemical tools allowed us to select the optimal (*i.e.* most active) chemical probes. We then successfully achieved a significant differential profile of tagged proteins between inhibitor probes and non-inhibitor controls and in competition experiments. After affinity chromatography, purified proteins were analyzed by LC-MS/MS and proteomic analysis allowed us to identify selectively enriched proteins, which are currently under validation. These proteins could then be further considered as new therapeutic targets enabling an indirect targeting of DNA methylation resulting in a better selectivity of DNA methylation inhibition in anticancer treatments.



## Short communication EpiChemBio 4

### Anti-Neoplastic Activity of Newly Synthetized DNMT inhibitors in Renal Tumors

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Renal Cell Cancer (RCC) comprises one of the 10 most common malignant neoplasms worldwide being the most mortal genitourinary cancer (Torre et al. 2015). Partial or total nephrectomy is the standard therapy for localized RCC, whilst for recurrent and/or metastatic disease the mainstream treatment is systemic therapy (Campbell et al. 2009; Posadas et al. 2017). Unfortunately, this therapeutic approach is not curative and eventually all patients will become resistant being the prognosis of metastatic RCC extremely poor, with 5-year survival rate of only 5-10% (Duran et al. 2016; Motzer and Russo 2000). Therefore, investigation on new compounds designed to directly target the molecular mechanisms involved in disease onset and progression is needed. It is widely accepted that epigenetic alterations are important drivers of renal tumor carcinogenesis (Jerónimo and Henrique 2014). In fact, several tumor suppressor genes were reported as inactivated by promoter hypermethylation in RCC, namely, genes encoding for *APC*, *CDH1*, *RARβ2*, *RASSF1A*, and *VHL* (Dulaimi et al. 2004; Hoque et al. 2004; Morrissey et al. 2001; Nojima et al. 2001). Thus, inhibiting the enzymes responsible for the establishment of DNA methylation marks, the DNA methyltransferases (DNMTs), might be of crucial relevance for RCC clinical management. Taking this into account, we studied four newly synthesized DNMT inhibitors derived from flavanones, in order to assess their anti-cancer and demethylating activity in RCC cell lines. All the four compounds were able to reduce tumor cell phenotype by diminishing cell viability and apoptosis' increase which were accompanied by cell morphology alterations and significant DNA damage. Moreover, these compounds were able to reduce global DNA methylations levels and DNMTs' expression. Additional studies are now mandatory to confirm these promising results to further evaluate the potential of these compounds in RCC therapy.

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## Short communication EpiChemBio 5

### Targeted DNA demethylation in human cells by 5-methylcytosine excision

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DNA methylation is a crucial epigenetic mark associated to gene silencing, and its targeted removal is a major goal of epigenetic editing. In animal cells, DNA demethylation involves iterative 5mC oxidation by TET enzymes followed by replication-dependent dilution and/or replication-independent DNA repair of its oxidized derivatives. In contrast, plants use specific DNA glycosylases that directly excise 5mC and initiate its substitution for unmethylated C in a base excision repair process. In this work, we have fused the catalytic domain of Arabidopsis ROS1 5mC DNA glycosylase (ROS1\_CD) to the DNA binding domain of yeast GAL4 (GBD). We show that the resultant GBD-ROS1\_CD fusion protein binds specifically a GBD-targeted DNA sequence in vitro. We also found that transient in vivo expression of GBD-ROS1\_CD in human cells specifically reactivates transcription of a methylation-silenced reporter gene, and that such reactivation requires both ROS1\_CD catalytic activity and GBD binding capacity. Finally, we show that reactivation induced by GBD-ROS1\_CD is accompanied by decreased methylation levels at several CpG sites of the targeted promoter. All together, these results show that plant 5mC DNA glycosylases can be used for targeted active DNA demethylation in human cells.

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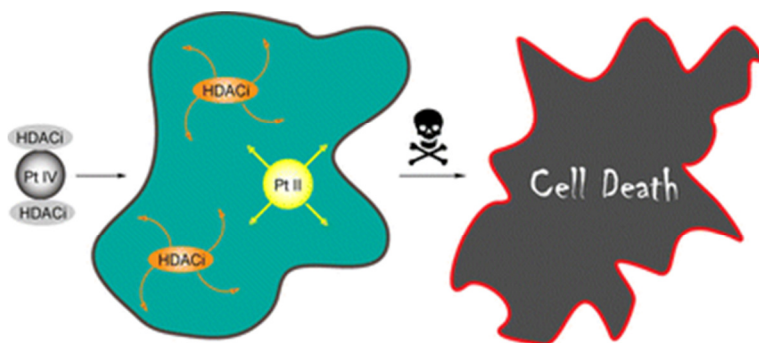
## Short communication EpiChemBio 6

### Epigenetic and antitumor effects of platinum(IV)-octanoato conjugates

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We present the anticancer properties of *cis*, *cis*, *trans*-[Pt(IV)(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(OA)<sub>2</sub>] [Pt(IV)diOA] (OA = octanoato), Pt(IV) derivative of cisplatin containing two OA units appended to the axial positions of a six-coordinate Pt(IV) center. Our results demonstrate<sup>1</sup> that Pt(IV)diOA is a potent cytotoxic agent against many cancer cell lines (the IC<sub>50</sub> values are approximately two orders of magnitude lower than those of clinically used cisplatin or Pt(IV) derivatives with biologically inactive axial ligands).

Importantly, Pt(IV)diOA overcomes resistance to cisplatin, is significantly more potent than its branched Pt(IV) valproato isomer and exhibits promising *in vivo* antitumor activity. The potency of Pt(IV)diOA is a consequence of several factors including enhanced cellular accumulation correlating with enhanced DNA platination and cytotoxicity. Pt(IV)diOA induces DNA hypermethylation and reduces mitochondrial membrane potential in cancer cells at levels markedly lower than the IC<sub>50</sub> value of free OA suggesting the synergistic action of platinum and OA moieties. Collectively, the remarkable antitumor effects of Pt(IV)diOA are a consequence of the enhanced cellular uptake which makes it possible to simultaneously accumulate high levels of both cisplatin and OA in cells. The simultaneous dual action of cisplatin and OA by different mechanisms in tumor cells may result in a markedly enhanced and unique antitumor effects of Pt(IV) prodrugs. tumors accessible by fiber-optic devices, thereby also decreasing the damage to normal, noncancerous cells.

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## **Short communication EpiChemBio 7**

### **The relation between famine exposure and schizophrenia explained by cross-chromosome regulation of *DUSP22* promotor hypermethylation**

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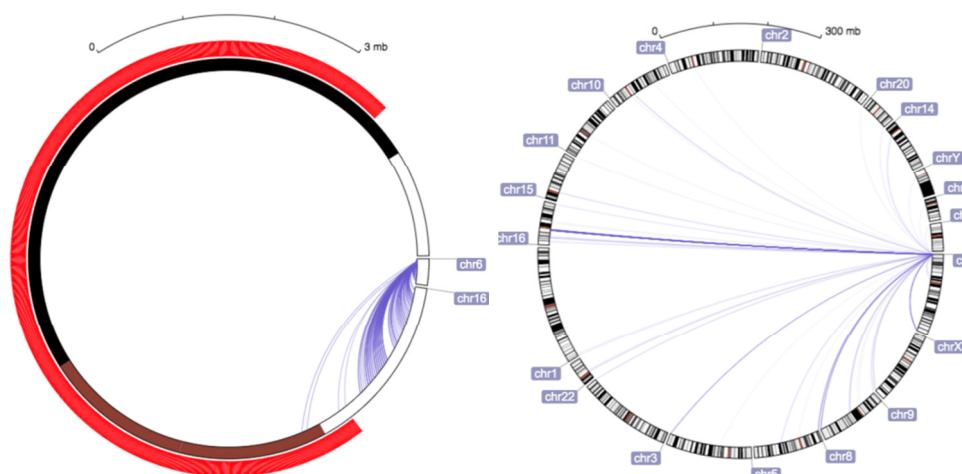
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## Summary

Epigenetic changes may account for the doubled risk to develop schizophrenia in individuals exposed to famine in utero. We therefore investigated DNA methylation in a unique sample of patients and healthy individuals conceived during the great famine in China. Subsequently we examined two case-control samples without famine exposure in whole blood and brain tissue. To shed light on the causality of the relation between famine exposure and DNA methylation we exposed human fibroblasts to nutritional deprivation. In the famine exposed schizophrenia patients we found significant hypermethylation of the dual specificity phosphatase 22 (*DUSP22*) gene promoter (Chr6:291687-293285) (N=153,  $p=0.01$ ). In this sample *DUSP22* methylation was also significantly higher in patients independent of famine exposure ( $p=0.025$ ), suggesting that hypermethylation of *DUSP22* is also more generally involved in schizophrenia risk. Similarly, *DUSP22* methylation was also higher in two separate case-control sample not exposed to famine using DNA from whole blood (N=64,  $p=0.03$ ) and postmortem brains (N=214,  $p=0.007$ ). *DUSP22* methylation showed strong genetic regulation across chromosomes by a region on chromosome 16 which was consistent with new 3D genome interaction data. Nevertheless, the presence of a direct link between famine and *DUSP22* transcription was supported by data from cultured human fibroblasts that showed increased methylation ( $p=0.048$ ) and expression ( $p=0.019$ ) in response to nutritional deprivation (N=10). Together these results point to an involvement of *DUSP22* in the molecular mechanisms underlying the increased risk of schizophrenia in response to in-utero famine and suggest a novel mechanism of how genes and the environment can interact.

**Fig. 1:** Overview of the chromosome-chromosome interactions measured with in situ Hi-C. Figure A zooms into the *DUSP22* DMR, while figure B provides an overview of the chromosome interactions. A darker blue indicates more frequent interactions.



## Short communication EpiChemBio 8

### Targeting protein lysine acetylation in inflammatory lung diseases

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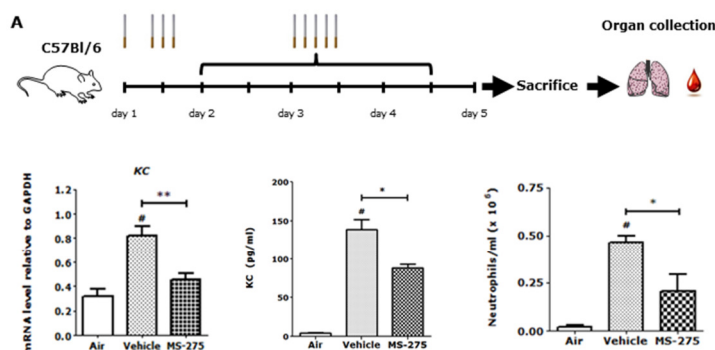
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**Objective:** Chronic inflammatory diseases, such as, for example, asthma, afflict millions of people worldwide. Molecular mechanisms regulating these diseases involve, among others, reversible protein lysine acetylations. Acetylations of histones and non-histone proteins proved to be crucial for regulation of nuclear factor  $\kappa$ B (NF  $\kappa$ B) mediated signalling. Targeting lysine acetyl transferases (KATs) and lysine deacetylases (KDACs) with innovative small molecule inhibitors provides chances for development of anti-inflammatory therapeutics.

**Methods/results:** We developed innovative HDAC inhibitors by integration photo-switches in their molecular structure, which allows for local activation of HDAC inhibition [1]. For the HDAC1, 2 and 3 selective HDAC inhibitors, Entinostat, we were able to identify anti-inflammatory effects in airway inflammation that were connected to up-regulation of the anti-inflammatory cytokine interleukin 10 and binding of the p65 subunit to the interleukin 10 promotor [2].

**Conclusions:** Altogether these studies demonstrate that targeting lysine acetylation and deacetylation has potential for inflammatory lung diseases.



**Figure 1:** Pharmacological testing of the HDAC inhibitor Entinostat in a model system for airways inflammation. Entinostat treatment via nebulization caused a decrease in the mRNA and protein levels of KC (mouse IL8) and the neutrophil count in lung.

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## Short communication EpiChemBio 9

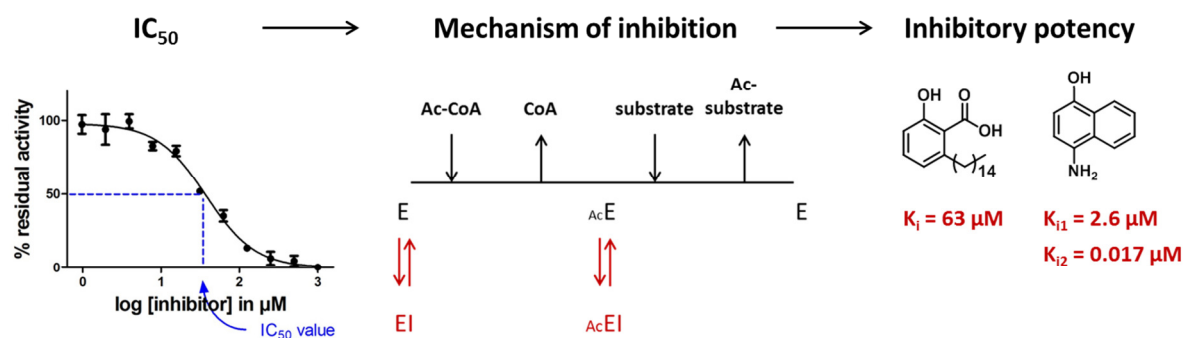
### Challenges in targeting histone acetyltransferases – A KAT8 case story

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Histone acetyltransferases (HATs) are epigenetic enzymes involved in the regulation of gene transcription and are potential targets in disease. The development of bioactive molecules or molecular probes targeting these enzymes, such as inhibitors, has met many challenges<sup>1</sup>. One of these challenges is the lack of kinetic evaluation of the enzymes and their inhibitors. HATs catalyze the reaction between two substrates, which complicates the calculation of inhibitory potency values ( $K_i$  values) from their observed 50% inhibitory concentration ( $IC_{50}$ ) in biochemical assays. Since the  $IC_{50}$  values depend on assay conditions, it is not possible to use these values for reproducible determination of the potency and selectivity. Therefore, using biochemical and biophysical methods, the  $K_i$  values of two structurally different inhibitors for the enzyme lysine (K) acetyltransferase 8 were determined<sup>2,3</sup>.



**Figure 1:** The determination of  $K_i$  values for two structurally different KAT8 inhibitors

The  $K_i$  values showed striking differences in potency and inhibitory mechanism between both inhibitors, which could not be revealed by the  $IC_{50}$ . The  $K_i$  values additionally revealed that the mechanism of inhibition could have consequences for the inhibitor activity *in-vivo*. This shows that the  $IC_{50}$  does not give sufficient information on the inhibitory potency and that the  $K_i$  values should be determined in case of KAT8 and other HATs.

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## Short communication EpiChemBio 10

### *In silico* identification of Isoform-Selective Hdac Inhibitors

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The acetylation state of histones is reversibly regulated by two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Overexpression of HDAC specific isotypes in many cancer cell lines and tumor tissues have been reported<sup>1</sup>. Global loss of monoacetylation at Lysine number 16 of histone H4 was discovered to be the common hallmark of human cancer cells<sup>2</sup>. HDAC inhibitors are potent inducers of cell cycle arrest in transformed cells and their subsequent death via apoptotic, autophagic and reactive oxygen species (ROS)-mediated pathways. They are also found to decrease cell migration and angiogenesis by targeting non-histone proteins. Therefore, HDACs are promising therapeutic targets for cancer studies. One of the greatest concerns with the use of HDAC inhibitors is their ability to disrupt multiple pathways pointing to their lack of specificity to a target enzyme. This has contributed to the cytotoxicity observed in many of the clinical trials. Class I family (HDACs 1, 2, 3, 8) and Class IIb member (HDAC6) enzymes were used as target proteins. The amino acids in the catalytic channels of these isoforms were similar even though the overall sequence similarity is 16%.

Here, we attempted to design more potent and isoform selective HDACIs from commercially available 2.7 million compounds on Otava database. The database was prescreened to obtain 200, 607 active compounds against HDACs 1,2,3,6 and 8. Using structure based virtual screening in Autodock vina 45 isozyme selective inhibitors were identified. Docking of selective inhibitors against their respective HDAC isoform and ADMET prediction resulted 40 best inhibitors; 10 for HDAC1, 10 for HDAC2, 8 for HDAC3, 4 for HDAC6 and 8 for HDAC8.

Interestingly, of all the 40 compounds identified, compound 33 was found to have the highest binding affinity and showed high selectivity for HDAC8. Compound 33 is a dicarboxylic acid derivative with a bulky cap (substituted phenanthrenyl moiety) and an aromatic linker. One carboxyl group interacted with TRY98 and PRO22 and the other interacted with TYR293 and MET261 via hydrogen bonds at the bottom of the pocket (**Figure 1**). In addition, various more interactions formed along the entrance and the interior of the channel resulted in the complete burial of compound 33 in HDAC8 catalytic channel. HDAC8 exhibits an acetate release channel different from that of HDAC1–3, and a unique lateral internal channel and these features could be exploited to achieve isoform selectivity<sup>3</sup>.



**Figure 1:** 3D representation of a complex of HDAC8 with compound 33

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## Short communication EpiChemBio 11

### Revisiting non-ribosomal macrocyclic peptides: Discovery of class I HDAC inhibitors with picomolar affinities

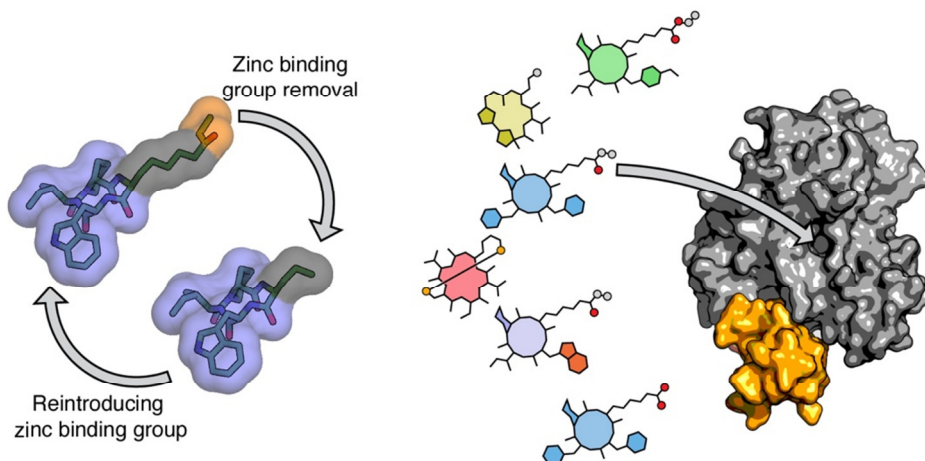
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Histone deacetylases (HDACs) are validated targets for treatment of certain cancer types and play numerous regulatory roles in biology, ranging from epigenetics to metabolism. Small molecules are highly important as tool compounds to probe these mechanisms as well as for the development of new medicines. Therefore, detailed mechanistic information and precise characterization of the chemical probes used to investigate the effects of HDAC enzymes are vital. Several cyclic tetrapeptide and depsipeptide natural products have proven useful as biological probes and drug candidates due to their potent activities as HDAC inhibitors. By interacting with both a catalytic zinc-atom and the surface of the HDAC enzyme, these natural products generally exhibit potent HDAC inhibition.<sup>1</sup>

We have interrogated Nature's arsenal of macrocyclic non-ribosomal peptide HDAC inhibitors by chemical synthesis and evaluation of more than 30 natural products and analogs. This furnished surprising trends in binding affinities for the various macrocycles, which were then exploited for design of highly potent class I and IIb HDAC inhibitors. Furthermore, thorough kinetic investigation revealed unexpected inhibitory mechanisms of important tool compounds as well as the approved drug Istodax (romidepsin). This work provides novel inhibitors with varying potencies, selectivity profiles, and mechanisms of inhibition and, importantly, affords insight regarding known tool compounds that will improve interpretation of their effects in biology and medicine.



**Figure 1:** Macrocyclic inhibitors of HDACs.

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## Short communication EpiChemBio 12

### Erasing the eraser: Treating parasitic disease with epigenetic

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Schistosomiasis, also called bilharzia, is a neglected tropical disease caused by a parasitic worm of the genus *Schistosoma*. Reported in 78 countries, this parasitic disease affects over 200 million people worldwide and cause more than 300 000 death every year [1], ranking it at the second position of the world's parasitic diseases. Currently the control and the cure of the disease rely on an intensive use of praziquantel, the only drug available. Despite its efficiency on every species of *Schistosoma*, the limited availability of the drug and its inefficiency to prevent second infection are a major problem of this strategy. Moreover, as a consequence of the lack of alternative, reduced efficiency and drug resistance start to be reported. Therefore the development of a new therapy is essential.

The A-ParaDDisE project is an international collaboration that aims to develop optimized epigenetic inhibitors for further testing and optimisation as drug candidates against the four mains parasitic diseases: malaria, schistosomiasis, leishmaniasis and Chagas disease.

Investigation on the *Schistosoma mansoni* worm led to the identification and the isolation of three class I histone deacetylase (HDAC)[2], a class of enzyme that remove the acetyl group from an N-acetyl lysine amino acid on a histone in the nucleosome. Among these three, *Schistosoma mansoni* histone deacetylase 8 (SmHDAC8) was observed at every stage of the life cylce and was the most expressed smHDAC. Further studies showed that SmHDAC8 plays an important role in the parasite infectivity[3] and that HDAC inhibitors induce mortality and apoptosis in schistosomula[4].

Targeting SmHDAC8 by synthetising selective inhibitors could therefore be a way to develop a new therapy for Schistosomiasis.

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## Short communication EpiChemBio 13

### Design and synthesis of novel Histone Deacetylase 6 zinc binding groups

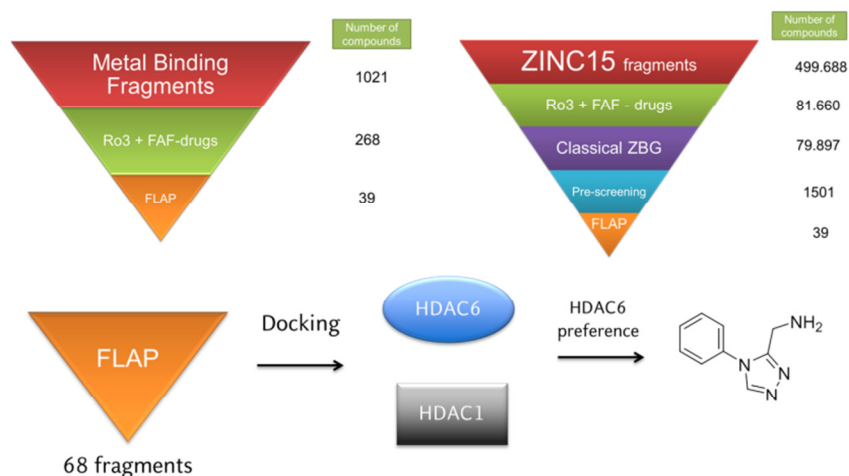
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Epigenetic alterations such as DNA methylation and post-translational histone acetylation are the most prevalent alterations of the genome. Histone Deacetylases (HDAC) are vital in this process and therefore attractive therapeutic targets. Eleven zinc dependent HDACs isoforms are expressed in humans, sharing a highly conserved catalytic domain [1]. Among them, HDAC6 is important for a wide range of diseases, due to its unique structural features and physiological functions and involved in the chemo-sensibilization [2]. Recently the crystal structure of human HDAC6 catalytic domain II revealed a wide solvent exposed binding site flanked by a large groove (basin), believed to act in the substrate recognition. These structural features can be used to design HDAC 6 selective inhibitors [3]. The classical HDAC inhibitors are divided in three parts: a cap group which interacts with the surface of the binding pocket, a linker and a zinc binding group (ZBG). Although hydroxamic acids (HA) are the dominant class of ZBG, they do not display direct influence in the isoform selectivity, which is known for other ZBGs such as 2-aminoanilides (HDAC1-3), 5-trifluoromethyl-1,2,4-oxadiazole-ZBG (class IIa), and 3-hydroxypyridin-2-thione-ZBG (HDAC6 and 8) [1]. We therefore performed a series of structure-based and ligand-based virtual screenings in two fragment libraries in order to identify novel fragments with potential selectivity against HDAC6. The best fragments were then selected for docking studies. Finally, the most promising fragments were selected and are currently being synthesized (Figure 1).



**Figure 1:** Overall scheme of the fragment-based design of HDAC6-selective novel ZBG

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## Short Communication EpichemBio 14

### Double life of the multifunctional disordered Tppp/p25:

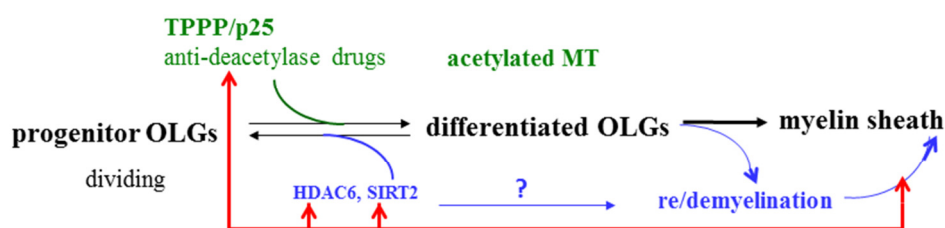
#### Physiological Function

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The cytoskeletal microtubule (MT) network exerts sensing, integrating and coordinating functions by its decoration with proteins/enzymes and posttranslational modifications. Our research group discovered a new brain-specific, multifunctional protein, denoted: [Tubulin Polymerization Promoting Protein/p25 \(TPPP/p25\)](#), which is predominantly expressed in oligodendrocytes (OLGs) of normal brain; its expression is crucial for the differentiation of OLGs, which are the major constituents of the myelin sheath. In fact, TPPP/p25 plays crucial role in controlling of the physiological and pathological functions of the MT network by its MT bundling and acetylation enhancing activities. We have shown that the disordered TPPP/p25 displays concentration-dependent dimerization<sup>1</sup> stabilized by disulfid bridges, that is promoted specifically by the bivalent zinc cation and GTP. The self-protein-protein interactions of TPPP/p25 result in structural changes coupled with enhanced tubulin polymerization activity; in this process the flexible core segment of TPPP/p25 plays determining role<sup>2</sup>.



**Figure 1:** Interactions affecting the maturation of oligodendrocytes (OLGs) leading to myelin sheath formation.

In addition to tubulin, the major physiological partner of the protein, TPPP/p25 interacts with atypical histone deacetylases HDAC6 and SIRT2, which regulate the acetylation level of tubulin by removing acetyl groups. The reversible post-translational acetylation of  $\alpha$ -tubulin at residue Lys-40 modifies the dynamics of the MT system. HDAC6 and SIRT2 are selectively inhibited by TPPP/p25, consequently the protein increases the intracellular acetylated tubulin level. Since the acetylation level of the MT network plays a crucial role in physiological processes, such as MT growing, cell differentiation, motility and cell cycle, HDAC6 and SIRT2 are objects of extensive pharmacological investigations. Tubulin deacetylase inhibitors are potential drug targets for several disorders (cancer, neurodegenerative, immunologic, metabolic, inflammatory and cardiovascular disorders). Within the frame of the COST project CM1046 EPICHEM our research group has been involved in the “anti-cancer drug discovery” projects by collaborating with the Jung’s and Bertrad’s labs.

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## Short communication EpiChemBio 15

### From DNA methyltransferase transition state analogues to chemical scaffolds for the inhibition of PRMT4

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RNA, DNA and histones methylation is implicated in various human diseases such as cancer or viral infections playing a major role in cell processes regulation especially in modulation of gene expression [1]. Methylation of deoxycytidines (dC) occurs at CpG sites, which are grouped in islands and essentially located in promoters, repeated sequences and CpG island shores. The enzymes responsible for DNA methylation are the DNA methyltransferases (DNMTs) that add a methyl group on the carbon-5 position of the deoxycytidine at the CpG site in the DNA by using S-adenosyl-L-methionine (AdoMet/SAM) as methyl donor [2]. Former DNMT2, now, TRDMT1, structurally close to DNMTs, weakly methylates DNA at cytosine but rather methylates tRNA [3]. tRNA methylation is implicated in metabolic activity of cancer cells and its inhibition has anti-proliferative effects [4]. Histone methyltransferases (HMT) catalyse the transfer of one, two, or three methyl groups to lysine and arginine residues of histone proteins. As DNA methylation, histone methylation is involved in the control of gene expression and affect genomic stability. We focused on the protein arginine methyltransferases (PRMTs) [5]. Because alteration of DNA, histone and RNA methylation patterns are associated to cancer, they represent an attractive therapeutic strategy and there is an emerging interest in the discovery of new inhibitors of DNMTs, RNA MTases and PRMTs. In the case of transferases, a potent chemical strategy to design inhibitors is to use transition state analogues. Interestingly, DNMTs, RNA MTases and PRMTs all share the same methyl donor, the AdoMet/SAM, and, concerning the substrate that is methylated, cytosine, guanine and arginine share some common pharmacological features. Thus we developed a convergent synthetic pathway starting from a protected bromomethylcytosine derivative to synthesise transition state analogues of the DNA methyltransferases. This approach led to seven 5-methylcytosine-adenosine compounds that resulted, surprisingly, inactive against hDNMT1, hDNMT3Acat, TRDMT1, and other RNA human and viral methyltransferases. Interestingly, two compounds an inhibitory activity against PRMT4 in the micromolar range. Crystal structures showed that compounds bind to PRMT4 active site and occupy the S-adenosyl-L-methionine binding site and interact with the arginine substrate site. Interestingly, important structural switches are induced by the binding of the compounds and correlate to the magnitude of inhibition. These findings open new routes for the conception of new PRMT4 inhibitors based on the 5-methylcytosine scaffold.

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## Short communication EpiChemBio 16

### The quinazoline ring as a privileged scaffold in Epigenetic (Poly)Pharmacology: From dual G9a Methyltransferase/LSD1 Demethylase Inhibitors to selective LSD1 Inhibitors

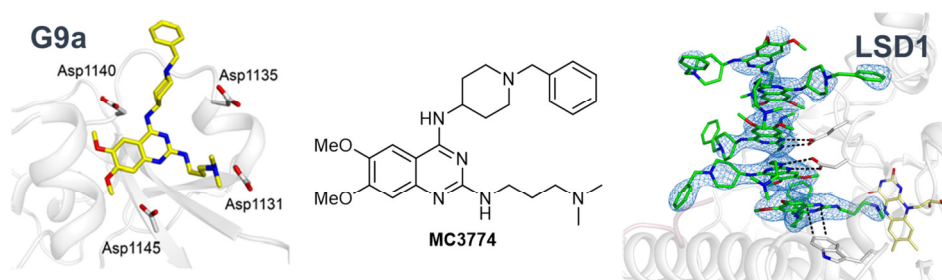
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Human histone methylation patterns result from the balance between lysine methylation and demethylation. The removal of methyl marks is performed by two distinct families of demethylases (KDMs) that differ for the reaction mechanism: LSD1/2 are FAD-dependent amine oxidases, while JmJc are Fe<sup>2+</sup>/α-ketoglutarate-dependent enzymes.<sup>1</sup> LSD1 is able to catalyze the demethylation of H3K4me1/2, plays a crucial role in the epigenetic modulation of gene expression and is considered a very promising target in anticancer chemotherapy.<sup>1,2</sup> Our investigation on quinazolines as H3K9 methyltransferase/demethylase or DNMT inhibitors led us to identify the compound MC3774 as a Lys-mimicking derivative that displays dual G9a methyltransferase/LSD1 inhibition in the (sub)micromolar range and it is endowed with a significant antiproliferative activity in human leukemia cells (MV4-11).<sup>3</sup> Anyway, by far the most interesting features of this compound are its different inhibition mechanisms.



**Figure 1:** Different binding modes of the dual inhibitor MC3774 with G9a (left side) and LSD1 (right side).

Despite designed by supposing that the dimethylaminopropyl chain could mimic the H3K4me2 moiety of the substrate within the LSD1 active site, MC3774 does not enter at all into the catalytic pocket but binds the enzyme disposing five copies of itself in a stacked way that obstruct the access to the active site. The orientation of the molecules is “face to face” and “head to tail”, and in both ways they interact with a cluster of negatively charged amino acidic residues.<sup>3</sup> This kind of non-covalent and reversible inhibition is typical for the interaction between quinazoline scaffold and the catalytic cleft of LSD1, in fact MC3774 does not inhibit G9a by using the same stacking mode, but by competing with the histone H3 substrate within the active site. On these bases, with the aim to increase the potency against both G9a and LSD1 or the selectivity versus LSD1, we prepared several analogs of the lead compound MC3774 by modifying the quinazoline scaffold at position 2 with alkylamino functions of different length variously substituted at omega position, by replacing the NH at position 4 with an oxygen atom or with N-CH<sub>3</sub>, or by modifying the N-benzyl moiety with other aryl-alkyl functions. The results led to more potent dual G9a/LSD1 inhibitors and to extremely potent and selective LSD1 inhibitors and were consistent with the distinct inhibition mechanisms.

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## Short communication EpiChemBio 17

### The Clinically Used Iron Chelator Deferasirox is an Inhibitor of Epigenetic JumonjiC Domain-Containing Histone Demethylases

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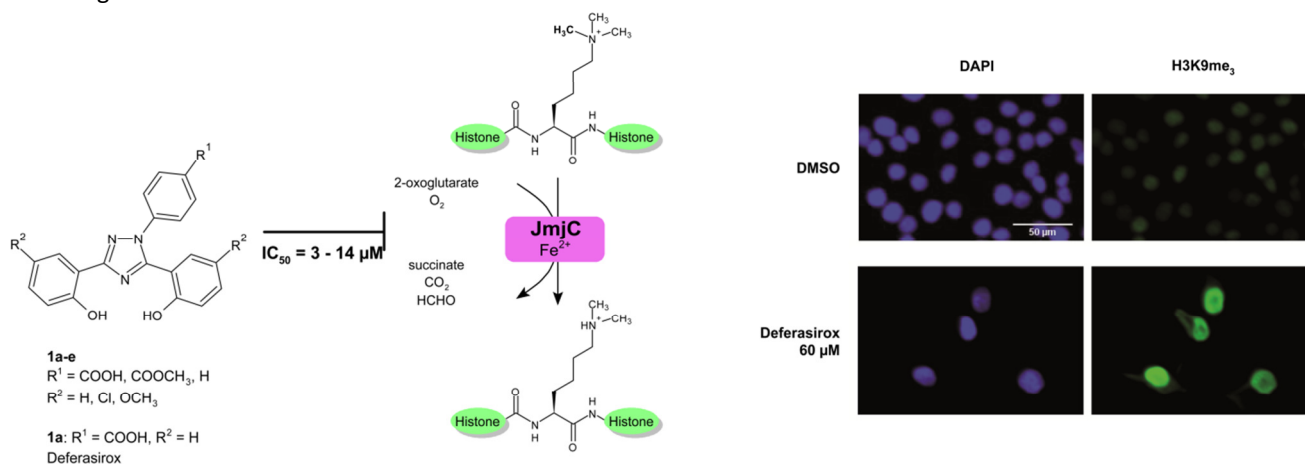
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Epigenetic mechanisms determining the cellular phenotype are maintained by chemical modifications both to DNA as well as to the proteins around which it is wrapped, the histones.<sup>[1,2]</sup> Iron(II)- and 2-oxoglutarate-dependent JumonjiC histone demethylases (JmJC KDMs) are epigenetic eraser enzymes, which oxidatively remove methyl groups from lysine residues in the histone tails and are implicated in gene regulation and manifestation of diseases, in particular cancers, making them viable drug targets.<sup>[2-7]</sup>

We have investigated a panel of clinically used iron chelators for their potential inhibition of JmJC KDMs and found them all to potently inhibit isolated JMJD2A (KMD4A), JARID1A (KDM5A), and JMJD3 (KDM6B) in *in vitro* assays. The mode of action was thoroughly investigated and revealed that one compound, deferasirox **1a**, is indeed a bona fide active site-binding inhibitor as demonstrated by enzyme kinetic studies, EPR and NMR spectroscopic evidence as well as docking.



Synthesis of derivatives with improved cell permeability showed significant upregulation of histone trimethylation in a cancer cell line and potent growth inhibition. Therefore, the beneficial therapeutic effects of deferasirox **1a** may involve epigenetic regulation through JmJC KDM inhibition. **1a** provides a useful starting point for the development of novel anticancer drugs targeting 2-oxoglutarate-dependent enzymes, while also raising concerns about potential adverse effects by modulating histone methylation in patients with an unrelated disorder, for whom this compound is currently in clinical use.

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**EpiChemBio (CM1406) and MuTaLig (CA15135) COST actions joint meeting 2017 Porto (PT), Sept 22-24 2017**

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## Short communication EpiChemBio 18

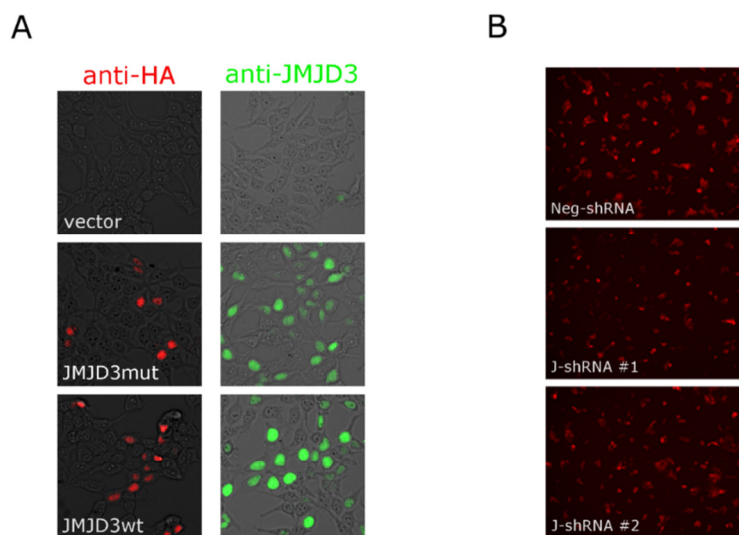
### Histone H3K27 Demethylase JMJD3 as a Therapeutic Target in Cancer

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My current project entails studying the function of jumonji domain-containing protein 3 (JMJD3) in cancer. In my previous publication I demonstrated that JMJD3 expression levels are reflected in changes in cancer cell phenotype and variations associated with cellular senescence, including senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity, the senescence-associated secretory phenotype (SASP), and changes in cytoskeletal structures, nuclear shape, and chromatin organization<sup>1</sup>. Expression of the SASP undermines normal tissue homeostasis and contributes to tumorigenesis and tumor progression<sup>2</sup>. JMJD3 exerts these actions through histone H3K27 (lysine 27 of histone H3) demethylase activity by removal of methyl groups from H3K27me3 (tri-methylated H3K27), as well as by direct effects on chromatin organization. The goal was to generate lentiviral particles capable of delivering DNA constructs to overexpress JMJD3 protein in cancer cells. Lentiviral transduction was efficient for producing stable cell lines for long-term studies (Figure 1A). The clinical significance of JMJD3 is that it is a key regulator of cytokine production in cancer. To explore JMJD3 as a novel therapeutic target in cancer to block the deleterious effect of the SASP, I used RNAi knockdown to inhibit JMJD3 expression/function (Figure 1B). These genetically modified cell lines can be used as tools for future studies that will examine the relationships between JMJD3 expression and molecules related to inflammaging (increased inflammation with aging) in cancer, such as cytokines, proteases, and growth factors. This provides me with a unique system to investigate the impact of chromatin structure, function and regulation on the aging/cancer relationship.



**Figure 1: Engineering of cell lines to study JMJD3 demethylase.** **A.** HEK293TN cells were used as target cells for testing lentiviral particles to overexpress functional wildtype and single point mutated (H1390A) catalytically inactive JMJD3 constructs: JMJD3wt and JMJD3mut. Each JMJD3 protein is fused to a hemagglutinin (HA) tag for easy detection. JMJD3 proteins were detected by immunocytochemistry using antibodies for HA and JMJD3 protein sequences. In addition, the empty vector backbone of the JMJD3mut construct was used as a control (without the JMJD3mut sequence portion). **B.** Targeting of JMJD3 in cells was achieved using pTRIPZ shRNA constructs purchased from Thermo Scientific (Waltham, MA). The pTRIPZ shRNA constructs are engineered to be Tet-On and produces tightly regulated induction of shRNA expression in the presence of doxycycline. In addition to driving expression of the shRNA, the promoter within these constructs also drives the expression of a TurboRFP reporter. HEK293TN cells were infected with lentiviral particles containing a control shRNA (Neg-shRNA) or two different shRNA constructs that target

JMJD3 (J-shRNA #1 and #2). Afterwards, cells were treated with doxycycline (2 µg/ml) at 24 and 48 hr to induce shRNA expression and turboRFP reporter activity was observed.

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## Short communication EpiChemBio 19

### BLIMP1 and EZH2 in antibody secreting malignancies

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BLIMP1 is a transcriptional regulator that associates with transcriptional co-regulators to regulate gene expression during development and differentiation of various different cell types. It is the master regulator of plasma cell differentiation as its expression in mature B-cells is sufficient to drive their differentiation to antibody secreting plasma cells [1]. BLIMP1 has been shown to be required for the survival of long-lived plasma cells as well as myeloma cells, but can conversely also work as a tumour suppressor in B-cell lymphoma[2][3]. Interestingly, in mouse primordial germ cells as well as the highly proliferative mouse pre-plasmablast, the BLIMP1 genome wide binding pattern overlap extensively with that of polycomb repressive complex 2 (PRC2), including its catalytic subunit EZH2 that catalyses the trimethylation of H3K27[4,5]. However, whereas BLIMP1 expression is high in non-proliferative normal plasma cells, EZH2 is repressed. Conversely, the two factors are co-expressed in myeloma cells as well as Waldenström's macroglobulinaemia (WM) cells. We therefore decided to investigate the potential interaction and collaboration between EZH2 and BLIMP1 in these two antibody secreting malignancies with the hypothesis that it could form the basis for the proliferative phenotype of the malignant cells, mimicking the pre-plasmablast proliferative phenotype. Despite evidence of DNA editing events, we repeatedly failed to obtain either viable U266 myeloma- or RPCI-WM1 WM cells using CRISPR-Cas9 targeting of EZH2 and BLIMP1. We therefore engineered RPCI-WM1 cells to contain doxycycline inducible microRNAs (miR) targeting the two factors, using two distinct targeting miRs for each factor. Subsequently, the knock down of either BLIMP1 or EZH2 caused rapid cell death of WM cells. We observed a significant increase in apoptotic cells 48h after induction and no viable cells remaining after 5 days. Concomitantly the mRNAs of several apoptotic mediators were de-repressed. Chromatin immunoprecipitation coupled with deep sequencing (ChIPseq) of BLIMP1 and H3K27me3, in WM cells revealed a significant overlap between the position of the polycomb mark and BLIMP1 binding, further strengthening our hypothesis that EZH2 and BLIMP1 co-operate on chromatin. Interestingly, when we chemically inhibited EZH2 function using EPZ-6438, we failed to observe cell death, whereas using astemizole to disrupt the PRC2 complex resulted in cell death, implying that a non-catalytic function of EZH2 is necessary for WM cell survival[6,7].

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## **Short communication EpiChemBio 20**

### **Induction of Antitumor and Antifungal agents by Epigenetic Modifiers in the endophytic fungal strain *Dimorphosporicola tragani*.**

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Fungal endophytes are known to produce a wide variety of secondary metabolites (SMs) involved in their adaptation and survival within higher plants. Plant-microbe interaction may influence the expression of some biosynthetic pathways, otherwise cryptic in these fungi when grown in laboratory conditions.

Epigenetic small-molecule modifiers of Histone Deacetylase (HDAC) and DNA methyltransferase (DNMT) activities have been successfully used to perturb the fungal secondary biosynthetic mechanisms, which has led to the induction of the expression of silent metabolite pathways. Adding epigenetic elicitors in fungal endophyte fermentations may induce the expression of biosynthetic pathways which may occur naturally in plant-microbe interaction.

The endophytic fungal strain *Dimorphosporicola tragani* isolated from the endemic plant *Arthrocnemum macrostachyum* from the saltmarsh of Cabo de Gata (Almeria) was shown to produce cytotoxic activity against a human tumor hepatocyte cell lines (HepG2). A systematic approach to enhance the activation of cryptic pathways by the use of epigenetic modifiers was applied to this strain.

The use of these epigenetic modifiers induced the production of three different antifungal and antitumoral activities. Induced antitumoral secondary metabolites were purified and identified by HPLC and LCMS/NMR techniques. Once purified, induced compounds were also identified and characterized in a panel of tumor cell lines.

## Short communication EpiChemBio 21

### Meta-analysis of commonly deregulated miRNAs in oral cancer

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Oral cancer is characterised by high mortality rate, low long-term survival rate and rising incidence among younger population.<sup>1</sup> Identification of deregulated miRNAs in oral cancer is important in searching for new sensitive molecular biomarkers with diagnostic, predictive and prognostic importance.<sup>2,3,4</sup> Development of high-throughput technologies enabled identification of differentially expressed miRNAs between cancer and non-cancerous tissue samples on a large scale. However, the technologies used in different studies are diverse and obtained results are inconsistent. The aim of the study was identification of commonly deregulated miRNAs between oral cancer and normal matched non-cancerous tissue by performing meta-analysis of published results on miRNA profiling in oral cancer. Meta-analysis included seven independent studies analysed by vote-counting method, followed by bioinformatic enrichment analysis. Across seven studies included in the meta-analysis, twenty miRNAs were found as commonly deregulated in oral cancer compared to non-cancerous tissue. Eleven miRNAs were consistently up-regulated in three or more studies (miR-21-5p, miR-31-5p, miR-135b-5p, miR-31-3p, miR-93-5p, miR-34b-5p, miR-424-5p, miR-18a-5p, miR-455-3p, miR-450a-5p, miR-21-3p) and nine were down-regulated (miR-139-5p, miR-30a-3p, miR-376c-3p, miR-885-5p, miR-375, miR-486-5p, miR-411-5p, miR-133a-3p, miR-30a-5p). Identified miRNAs meta-signature was functionally characterized by KEGG enrichment analysis. Twenty four KEGG pathways were significantly enriched in oral cancer miRNAs meta-signature and TGF-beta signaling was the top enriched signaling pathway. The largest number of meta-signature miRNAs was involved in sphingolipid signaling pathway. Natural killer cell mediated cytotoxicity was the pathway with the largest number of genes regulated by miRNAs reported in the current study. The rest of enriched pathways in our miRNA list describe different malignancies and signaling. Identified commonly deregulated miRNAs might be considered as a potential biomarkers for distinguishing oral cancer tissue from normal, non-cancerous tissue. Validation and mechanistic studies are warranted in order to confirm findings from the meta-analysis.

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## **Short communication EpiChemBio 22**

### **RESPONSE project: Chromatin regulators as biomarkers and combinatorial drug targets in colorectal cancer therapy.**

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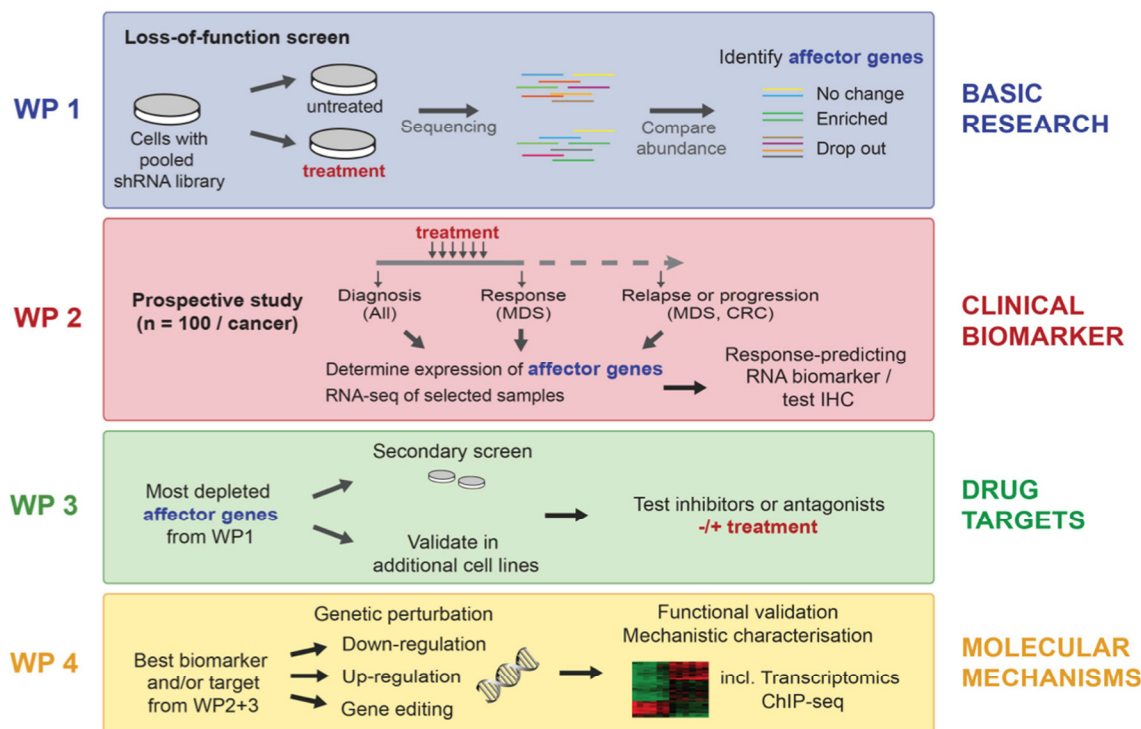
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Colorectal cancer (CRC) is the second most common cancer in Europe. Primary and acquired resistance to current treatment schemes is the cause for poor survival of one third of the patients that are diagnosed at an advanced stage of the disease<sup>1,2</sup>. Thus, development of response predicting biomarkers and improved therapies is urgently needed. Current therapies in advanced metastatic CRC are essentially based on the action of chemotoxic agents that either inhibit DNA synthesis or induce DNA damage<sup>3,4,5</sup>. Chromatin structure modulates access to DNA, therefore, manipulating chromatin-regulatory factors could contribute to increase effectiveness of cytostatic drugs. Furthermore, several chromatin factors are mutated or aberrantly expressed in tumors at variable frequencies<sup>6,7</sup>; those altered chromatin players could contribute to drug susceptibility. In order to identify which chromatin factors could be novel targets for overcoming resistance to chemotherapy, we will use a genetic loss-of-function screen in CRC cell lines. Comparing clonal selection in cells non-treated and treated with drugs that mimic therapeutic regimes in patients, will reveal which factors lead to sensitize or resistance. This information will be validated in primary samples, and predictive value will be assessed as well. The sensitizing genes will be evaluated as drug targets for combinatorial therapeutic approaches.

Our aim is to identify response-predicting biomarkers and novel drug targets among chromatin factors, that would be suitable for improved combinatorial therapeutic approaches. Funded by ISCIII, the RESPONSE project applies the same approach to three major cancers and their current best treatment. In addition to CRC treated with FOLFOX or FOLFIRI, this includes MDS treated with azacitidine, and non-small lung cancer treated with chemotherapy.



**Figure 1. Project summary:** it comprises four multidisciplinary work packages linking basic and clinical research. Our goal is to find chromatin biomarkers that predict response to treatment and that can be targeted by novel drugs. By combining basic and clinical research we aim to find novel more efficient therapeutic approaches to fight against chemoresistance.

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## Short communication EpiChemBio 23

### Studying the Epigenetic Mechanisms and Biomarkers in Neuropsychiatric Diseases

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#### Abstract

Our group studies the epigenetic mechanisms and look for biomarkers in several neuropsychiatric diseases. It is known that age-associated memory decline is due to variable combinations of genetic and environmental risk factors<sup>1</sup>. How these risk factors interact to drive disease onset is currently unknown. We have several research lines: First, we begin to elucidate the mechanisms by which post-traumatic stress disorder (PTSD) at a young age contributes to an increased risk to develop dementia at old age. We show that the actin nucleator Formin 2 (*Fmn2*) is deregulated in PTSD and in Alzheimer's disease (AD) patients. Young mice lacking the *Fmn2* gene exhibit PTSD-like phenotypes and corresponding impairments of synaptic plasticity while the consolidation of new memories is unaffected. However, *Fmn2* mutant mice develop accelerated age-associated memory decline that is further increased in the presence of additional risk factors and is mechanistically linked to a loss of transcriptional homeostasis<sup>2</sup>. Second, we study the role of IGF2/IGFBP7 signaling that regulates fear extinction and is altered in Alzheimer's disease, as a molecular link between psychiatric and neurodegenerative diseases<sup>1,3</sup>. Third, we try to find biomarkers of therapeutic efficacy for depression, schizophrenia and bipolar disorder studying the distribution of dopamine D2 and serotonin 5-HT2A receptor clusters, as well as monoamine transporters (MATs) in peripheral blood samples (lymphocytes) of healthy control subjects and patients before and after pharmacological treatment<sup>4</sup>. Our data present a new approach to explore the connection between AD risk factors across life span and provide mechanistic insight to the processes by which neuropsychiatric diseases at a young age affect the risk for developing dementia. Moreover, we try to find new clinical and biological markers of therapeutic efficacy and design therapeutic strategies to diagnose, prevent and treat different neuropsychiatric diseases.

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## Short communication EpiChemBio 24

### Environmental programming of respiratory allergy: utility of a child's spit epigenome

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Epigenetic DNA methylation changes can be part of the underlying molecular mechanisms leading to complex diseases. Early life exposures like parental lifestyle and exposure to chemicals can alter DNA methylation patterns, and thereby predispose the child to develop respiratory allergy (RA) later in life. Longitudinal birth cohorts are instrumental to study disease development, but DNA biomarker research is hampered because blood sampling is kept to a minimum for practical and ethical reasons. Saliva is a non-invasive and convenient source of DNA that can be used for biomarker research. In this study, we aimed at discovery and confirmation of differential methylation regions (DMR) in saliva of children with RA when comparing to controls.

Saliva samples collected in the two independent longitudinal birth cohorts (Flanders Environment and Health Surveys FLEHS1 & FLEHS2) were analysed using Illumina Methylation 450K BeadChips. A statistical analysis pipeline was developed in R to identify genome-wide differential methylation. We identified 23 DMRs in saliva from 11y old allergic children (self-reported/doctor's diagnosed RA, Phadiatop IgE  $\geq 0.35$  kU/L; N=26) vs. controls (no self-reported/diagnosed RA, Phadiatop IgE < 0.35 kU/L; N=20) in the FLEHS1 cohort. A set of 7 DMRs was selected for further validation by iPLEX MassArray analysis. First, iPLEX analysis was performed in the same 46 FLEHS1 samples that were previously analysed on the 450K methylation arrays, to allow technical validation. iPLEX results correlated significantly with the 450K methylation array data ( $P < 0.0001$ ), though iPLEX analysis confirmed 4 of the 7 identified DMRs in the FLEHS1 study.

Aiming for biological confirmation, we studied these DMRs in an independent birth cohort FLEHS2. Due to a lack of blood samples to measure IgE levels in the FLEHS2 cohort, cases and controls were identified as: 1) cases = doctor's diagnosed/self-reported RA symptoms ever (N=19); and 2) controls = no self-reported/diagnosed RA (N=20). When studying the 7 DMRs by means of iPLEX analysis in the FLEHS2 cohort, only a DMR in the *GLI2* gene showed a statistically significant difference in methylation between RA cases and controls. *GLI2* has a regulating role in IL4 signalling and can modulate T-helper differentiation and allergic disease, and might thus be an interesting DNA methylation marker to study for further biomarker development.

Interestingly, the RA-related hypermethylation in *GLI2* correlated significantly with life time exposures towards air pollution markers PM<sub>10</sub>, NO<sub>2</sub> and O<sub>3</sub>. Using the statistical framework developed by Valeri and VanderWeele (*Psychol Methods*, 2013), *GLI2* hypermethylation was observed to partially mediate the effects of PM<sub>10</sub>, NO<sub>2</sub> and O<sub>3</sub> on RA.

This project is providing novel insights in the molecular mechanisms that may predispose children to RA development. We are among the first to show the utility of saliva to identify DNA methylation marks in children that are relevant for RA.

## Short communication EpiChemBio 25

### May epigenetics explain the serine proteases involvement in grapevine resistance to *Plasmopara viticola*?

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Grapevine (*Vitis vinifera* L.), the most important fruit plant cultivated worldwide due to its economic importance in the wine industry, is highly susceptible to downy mildew, caused by *Plasmopara viticola*. The attack by this pathogen affects leaves, fruits and shoots, causing berry quality reduction and significant yield losses. Current prevention approaches to downy mildew disease control include extensive fungicide application each growing season, not always effective, very prejudicial to human health and with consequent impact in the economy and environment, so the search for alternative control methods is crucial.

Our recent results suggest that a family of serine peptidases, named subtilases (SBTs), may be involved in the establishment of immune priming and resistance mechanisms leading to the establishment of an incompatible grapevine-*Plasmopara viticola* interaction<sup>1,2</sup>. Indeed, these SBTs are constitutive expressed in resistant genotypes and highly induced after *P. viticola* inoculation<sup>1,2</sup>, thus the full comprehension of the mechanism of action of these proteins is very important.

One of our biggest goals is to understand how the subtilase genes are regulated and to uncover their action mechanisms. Epigenetics is an emerging field with high potential to understand gene regulation. Indeed, in *Arabidopsis thaliana*, it was observed that the SBT3.3 gene expression is under negative epigenetic control since its expression is relieved following inhibition of RNA-directed DNA methylation<sup>3</sup>. In grapevine, the epigenetic control of subtilase genes is still unknown. So, our first approach is to compare the promoter region of grapevine SBT genes searching for a conserved signature between some of the members of this family that may linked them to pathogen resistance. Promoter binding motifs and interaction molecules that regulate SBT expression may allow us to understand SBT role in this pathosystem.

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## Short communication EpiChemBio 26

### Epigenetic biomarkers for liquid biopsy-based testing of prostate cancer patients

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Liquid biopsy is a minimally invasive technology for detection of molecular biomarkers and a valuable alternative to surgical biopsies. Liquid biopsy-based detection of circulating tumor cells or cancer-derived nucleic acids opens a new way for non-invasive cancer diagnostics and follow-up of cancer patients. Prostate cancer (PCa) is the most frequent cancer among men in Europe with the highest mortality rates registered in the Baltic region, including Lithuania. Biomarkers for specific PCa detection and identification of most aggressive cases might improve the disease management and patients' survival. Based on the genome- and epigenome-wide analysis of PCa, our group has developed several informative and simple urine-based PCa-specific biomarker panels.

Quantitative analysis of more than 300 urine samples from PCa patients and controls revealed high sensitivity and specificity of DNA methylation-based<sup>1</sup> and miRNA-based<sup>2,3</sup> biomarker panels. A set of three hypermethylated genes (*RASSF1*, *RARB*, and *GSTP1*) in urine analysis showed sufficient diagnostic power and potential to predict biochemical progression after radical prostatectomy<sup>1</sup>. The combined analysis of two urinary miRNAs<sup>2</sup>, miR-148a and miR-375, was highly sensitive and specific for clinically significant PCa in two cohorts (AUC=0.79 and 0.84) and strongly improved the diagnostic power of the PSA test (AUC=0.85), including the diagnostic "grey zone" (AUC=0.90).

Several novel PCa-specific epigenetic biomarkers were identified utilizing global DNA methylation profiling of PCa tissues and were subjected to further gene-targeted analyses. The *PRKCB*, *CCDC181*, and *ADAMTS12* genes were frequently methylated in PCa tissues (N=129) and showed direct association with the lower expression levels of the respective transcripts. Moreover, methylation of the three genes was detectable in urine from PCa cases with the localized (N = 54) and advanced castration-resistant (N=82) disease suggesting a diagnostic and prognostic potential of these novel DNA methylation-based biomarkers.

In conclusion, urine-based molecular tests might be useful as a tool for non-invasive detection of PCa-derived cells. Epigenetic urine-based biomarkers may be utilized for non-invasive screening of men at high risk of PCa, to monitor the disease status in active surveillance groups, or to predict postsurgical PCa progression.

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## Short communication MuTaLig 1

### The role of the chromone scaffold in the development of novel multitarget agents in Alzheimer's disease

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Alzheimer's disease (AD) is an age-related neurodegenerative process characterized by a progressive memory loss, decline in language skills and other cognitive impairments. Although AD etiology is not yet known, protein aggregation, oxidative stress and low levels of acetylcholine seem to play a significant role in the disease pathophysiology.<sup>1</sup> Moreover, monoamine oxidase-B inhibitors (IMAO-B) are by now considered to be beneficial for neurodegenerative diseases' therapy, namely AD.<sup>2</sup>

Due to the pressing need for AD disease-modifying drugs, the development of multitarget-directed ligands (MTDLs) hitting druggable targets, namely acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and/or monoamine oxidase-B (MAO-B) based on novel scaffolds have been increasingly exploited.

Within this framework, our group has been focused on the validation of the chromone scaffold for the development of new drug candidates for neurodegenerative diseases.<sup>3, 4</sup> Accordingly, the present communication comprises the best chromone-based IMAO-B (effective in the nanomolar range) developed so far, comprising synthetic, biochemical and structural studies. Additionally, it will also be presented the development of novel dual-target inhibitors (operating in a submicromolar and low micromolar activity towards MAO-B and AChE).

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## Short communication MuTaLig 2

### Novel Multi-Target Ligands of aminergic GPCRs as potential antipsychotics

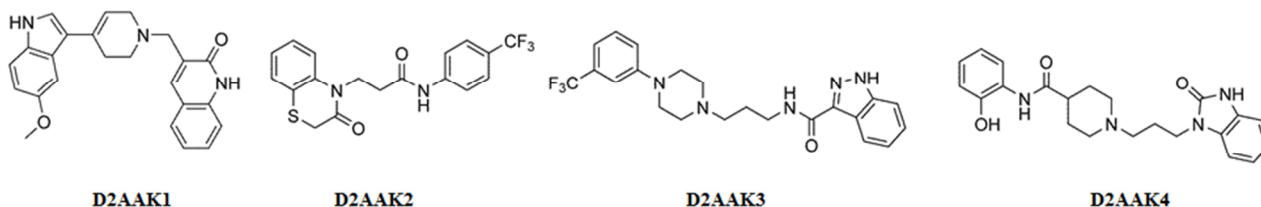
Agnieszka A. Kaczor<sup>a,b\*</sup>, Katarzyna M. Targowska-Duda<sup>c</sup>, Andrea G. Silva<sup>d</sup>, Marta Kruk-Słomka<sup>e</sup>, Peter Kolb<sup>f</sup>, Antti Poso<sup>b</sup>, Grażyna Biała<sup>e</sup>, Marian Castro<sup>d</sup>

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Structure-based virtual screening using a D<sub>2</sub> receptor homology model was performed to identify D<sub>2</sub> receptor ligands as potential antipsychotics.<sup>1</sup> As a result of a screen of a library of 1.6 million compounds, we selected 21 compounds, which were subjected to experimental validation. From 21 compounds tested, we found ten D<sub>2</sub> ligands (47.6% success rate, among them D<sub>2</sub> receptor antagonists as expected) possessing additional affinity to other receptors tested, in particular to 5-HT<sub>1A</sub> (partial agonists) and 5-HT<sub>2A</sub> receptors (antagonists). The affinity of the compounds ranged from 58 nM to about 24 μM. Similarity and fragmental analysis indicated a significant structural novelty of the identified compounds. We found one D<sub>2</sub> receptor antagonist that did not have a protonatable nitrogen atom which is a key structural element of the classical D<sub>2</sub> pharmacophore model necessary to interact with the conserved Asp(3.32). This compound exhibited over 20-fold binding selectivity for the D<sub>2</sub> receptor compared to the D<sub>3</sub> receptor. We provide additional evidence that the amide hydrogen atom of this compound forms a hydrogen bond with Asp(3.32) by testing its derivatives which cannot maintain this interaction. We confirmed antagonistic/partial agonistic/agonistic properties of the compounds towards the receptors in *in vitro* assays and in *in silico* studies as the ligands affect the ionic lock interaction. The four best compounds (D2AAK1-D2AAK4) were subjected to *in vivo* evaluation.<sup>2</sup> All the compounds decreased amphetamine-induced hyperactivity (when compared to the amphetamine-treated group), measured as spontaneous locomotor activity in mice. In addition, a passive avoidance test demonstrated that all the compounds improved memory consolidation after acute treatment in mice. Elevated plus maze tests indicated that all the compounds induced anxiogenic activity 30 minutes after acute treatment. 60 minutes after administration D2AAK1 displayed anxiolytic activity, D2AAK3 lack of activity and the anxiogenic activity of D2AAK2 and D2AAK4 was still observable. In order to optimize the structures of the lead compounds, we designed, synthesized and tested their modifications.



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### Short communication MuTaLig 3

## From Cholinesterase Inhibitors to Multifunctional Anti-Alzheimer's agents with Anti-Aggregating properties

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The multi-target-directed ligand design strategy is a way of searching for new effective drugs in the treatment of diseases caused by complex pathomechanisms, such as the Alzheimer's disease. Our research focuses on multifunctional ligands that influence cholinesterases as a symptomatic target and amyloid beta (A $\beta$ ) and tau protein as a disease-modifying target. At the beginning we were interested in cholinesterase inhibitors, which were able to ameliorate cholinergic neurotransmission and improve learning and memory functions. Target compounds were designed as dual binding site inhibitors able to interact with the catalytic site of the enzyme, and also able to bind at the peripheral active site of acetylcholinesterase (AChE) responsible for AChE-induced A $\beta$  aggregation. These studies led to the identification of some leads with multi-target activity, including anti-cholinesterase inhibitory potency, A $\beta$  anti-aggregation activity, neuroprotective effect against A $\beta$  toxicity, and beneficial effects on memory *in vivo*.<sup>1</sup> Later on, we have developed novel multifunctional ligands as potential inhibitors of AChE and/or butyrylcholinesterase (BuChE) and inhibitors of the enzyme  $\beta$ -secretase (BACE1). As a result of these studies we have discovered a multifunctional agent with moderate inhibitory activities towards both enzymes; BuChE (IC<sub>50</sub> = 7.12  $\mu$ M) and BACE1 (IC<sub>50</sub> = 79.68  $\mu$ M), endowed with A $\beta$  anti-aggregation activity (21.8% at 10  $\mu$ M).<sup>2</sup> Currently, we focus on the design and synthesis of multifunctional molecules that lower the production of A $\beta$  as BACE1 inhibitors as well as inhibit the aggregation of neurotoxic peptides, such as A $\beta$  and tau protein. Among the novel series of compounds, we identified inhibitors of both enzymes, BuChE and BACE1, endowed with anti-aggregating properties against A $\beta$  and tau protein. The most potent agent was characterized by IC<sub>50</sub>*eq*BuChE = 2.92  $\mu$ M, IC<sub>50</sub>*h*BuChE = 5.74  $\mu$ M, IC<sub>50</sub>*h*BACE1 = 41.60  $\mu$ M, IC<sub>50</sub>aggr.A $\beta$  = 3.09  $\mu$ M, *htau*244-372 aggr. inh = 54% at 10  $\mu$ M and good penetration into the brain.

#### Acknowledgments

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## Short communication MuTaLig 4

### Targeting A<sub>1</sub> or/and A<sub>2A</sub> Adenosine Receptors and MAO-B to treat neurodegenerative diseases

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The actions of adenosine are mediated by four adenosine receptor (AR) subtypes, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>, all of which couple to G proteins [1]. The A<sub>1</sub> ARs are widely expressed throughout the human brain including areas important for cognitive function. A<sub>1</sub> AR antagonists enhance the release of a number of neurotransmitters e.g. acetylcholine, glutamate, serotonin and norepinephrine. Antagonists of A<sub>1</sub> ARs have been suggested as potential treatment of cognitive deficits associated with Alzheimer's (AD) and Parkinson (PD) diseases. A<sub>2A</sub> ARs are localized exclusively in the dopamine enriched areas, and are co-localized with dopamine D<sub>2</sub> receptors. Blockade of A<sub>2A</sub> receptor potentiates D<sub>2</sub> receptor-mediated neurotransmission and therefore reduces the effects of striatal dopamine depletion in PD. This provides the basis for a role of A<sub>2A</sub> receptors antagonists in the treatment of PD. Monoamine oxidase B (MAO-B) is an enzyme which catalyzes the oxidative deamination of dopamine; inhibitors of this enzyme may slow the depletion of dopamine stores in PD brain and therefore MAO-B inhibitors are useful in the treatment of PD. Targeting A<sub>1</sub> or/and A<sub>2A</sub> ARs and MAO-B together may be beneficial in the treatment of neurodegenerative diseases such as AD and PD. In our efforts to develop potential drugs for the treatment of neurodegenerative disorders annelated derivatives of xanthines with substituted N-benzylamine moiety were designed and synthesized. Such compounds were compared with substituted 8-benzylamine-substituted xanthine derivatives. Compounds were evaluated for their activity against A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> ARs. Inhibitory activity towards MAO-B was determined by commercially available human recombinant MAO-B expressed in baculovirus infected insect cells using the Amplex Red monoaminoxidase assay kit (Life Technologies). The antioxidant activity of the compounds was additionally measured using the FRAP method based on the compounds' ability to reduce ferric to ferrous ions at low pH. Results were compared to the reference compound ascorbic acid and expressed as % of ascorbic acid activity. A<sub>1</sub>/A<sub>2A</sub> AR ligands with K<sub>i</sub> values in the nanomolar range were identified combined with MAO-B inhibiting activity (IC<sub>50</sub> values also in the submicromolar range). Structure-activity relationship analyses showed that the activity depends on the substituted benzylamine moiety and the way of (non)annelation of the xanthine. Drug-like properties of the examined compounds were estimated *in silico*.

Financial support by the Jagiellonian University Medical College grant no. K/ZDS/007121, National Science Center grant based on decision No DEC- NCN-DEC-2012/04/M/N24/00219 and the MuTaLig COST Action (CA15135) are gratefully acknowledged.



## Short communication MuTaLig 5

### Crosstalk between PDK1 and AURORA KINASE A: development of small multi-target agents

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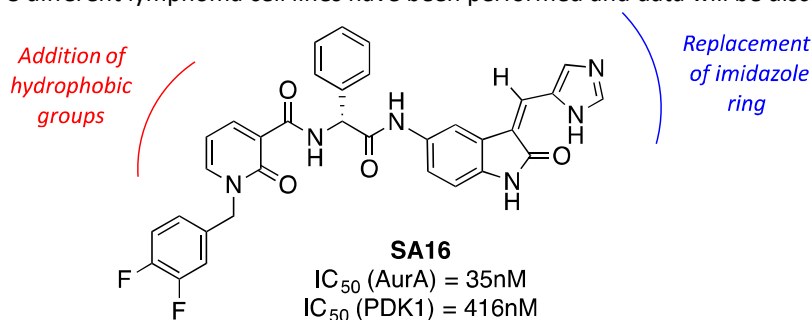
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The PI3K/PDK1/Akt signaling axis is intensely involved in inhibition of apoptosis and stimulation of cell proliferation. The serine/threonine kinase PDK1 acts as one of the main mediators of this pathway. Among the great number of other key signaling pathways interacting with PI3K/PDK1/Akt, Aurora A kinase is involved in pro-oncogenic signaling through both mitotic and non-mitotic functions.

Consistently, our main goal was to design and synthesise new small multi-target molecules capable to inhibit both *Aur-A* and *PDK1* kinases. Our efforts allowed us to identify SA16, a new molecule that showed potency values against isolated *Aur-A* and *PDK1* of 35nM and 416nM, respectively <sup>1</sup>. The dual inhibitor SA16 induces GBM cell proliferation, reduces tumor invasiveness and triggers apoptosis. Most importantly, SA16 showed an increased efficacy against GSCs, promoting their differentiation and apoptosis <sup>1</sup>. The effects induced by SA16 were entirely comparable to the co-administration of PDK1 and *AurA* inhibitors (MP7 and Alisertib, respectively).

These results prompted us to develop new SA16 analogues with an improved PDK1 potency and a retained *Aur-A* inhibitor activity. The chemical manipulations carried out on SA16 skeleton consisted in a) the addition of hydrophobic groups on pyridonyl nucleus, b) the replacement of imidazole ring with basic moieties. The new compounds were evaluated for their activity against both kinases. Some of them showed a more balanced profile than SA16. Further in vitro investigations in 8 different lymphoma cell lines have been performed and data will be discussed.



**Figure 1:** MedChem optimization on SA16.

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## Short communication MuTaLig 6

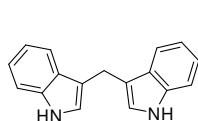
### Diindolylmethanes (DIMs) as novel anti-cancer agents targeting GPR84 and cannabinoid receptors

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3,3'-Diindolylmethane (DIM) is a dietary indole derived from digestion of indole-3-carbinol, found in cruciferous vegetables such as broccoli, brussel sprouts, cabbage, and cauliflower. DIM has been and still is evaluated in clinical trials for various forms of cancer due to its promising anti-tumor effects demonstrated in preclinical *in vitro* and *in vivo* studies. Moreover; DIM is being investigated for the treatment of viral and bacterial infections and it is currently used to treat recurrent respiratory papillomatosis, a disease caused by the human papilloma virus, because of its immunomodulatory effects. The cellular effects of DIM are numerous, however only few molecular targets of DIM have been established, so far. DIM has been reported to directly bind and activate the arylhydrocarbon receptor (AhR), which may be associated with gastric carcinogenesis. By binding to the estrogen receptor, DIM inhibits estrogen-induced growth of breast cancer cells. Recently, DIM was identified as the first synthetic agonist of the immunostimulatory orphan G protein-coupled receptor (GPCR) GPR84.<sup>1</sup> The expression of GPR84 in the peripheral immune system and in microglia suggests an immunomodulatory role. Structure-activity relationship analysis of DIM derivatives by our group led to the development of new agonists of GPR84 with improved potency (for example **PSB-16671**, EC<sub>50</sub> 41.3 nM).<sup>2</sup>

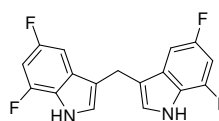


**DIM**

human GPR84; EC<sub>50</sub> = 252 nM<sup>2</sup>

human CB<sub>1</sub>; K<sub>i</sub> = 5420 nM

human CB<sub>2</sub>; K<sub>i</sub> = 690 nM



**PSB-16671**

human GPR84; EC<sub>50</sub> = 41.3 nM<sup>2</sup>

human CB<sub>1</sub>; K<sub>i</sub> = 2640 nM

human CB<sub>2</sub>; K<sub>i</sub> = 1100 nM

#### 3,3'-Diindolylmethane (DIM) derivatives as multitarget drugs (K<sub>i</sub> values determined in our group)

DIM has also been reported to activate another class of GPCRs, the cannabinoid (CB) receptors CB<sub>1</sub> and CB<sub>2</sub>.<sup>3</sup> The CB<sub>1</sub> receptor is mainly expressed in the cells of central nervous system (CNS), while CB<sub>2</sub> is predominantly expressed in the immune system, for example in the tonsils and spleen, and in microglia, similarly to GPR84. Recent evidence emerges that CB receptors are often overexpressed in tumor cells, and could therefore be used as novel targets for cancer. Moreover, several cannabinoid agonists were reported to inhibit tumor growth. In an effort to develop potent ligands for multiple targets, a library of DIM derivatives synthesized in our group was screened at CB receptors. As a result, we identified compounds that were able to activate both CB<sub>1</sub> and CB<sub>2</sub> receptors in the low micromolar range. We therefore propose that DIM could be a valuable scaffold for a multi-target approach, activating GPR84 thereby stimulating the immune system, and at the same time activating CB receptors which is expected to result in an anti-cancer effect and to reduce pain.

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## Short communication MuTaLig 7

### ABAD (17 $\beta$ -HSD10) inhibitors and their effect on key mitochondrial enzymes in the research of neurodegenerative diseases or cancer treatment

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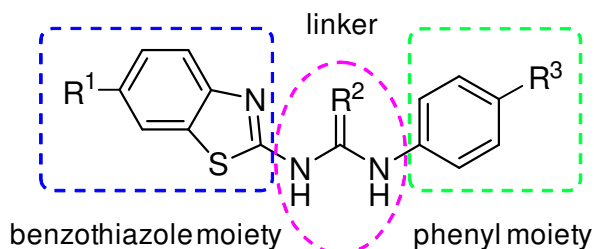
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Mitochondrial amyloid-binding alcohol dehydrogenase (ABAD), also known as 17 $\beta$ -hydroxysteroid dehydrogenase type 10 (17 $\beta$ -HSD10), has been recognized to interact with amyloid-beta peptide (A $\beta$ ), which may lead to pathological changes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



**Figure 1:** General structure of ABAD (17 $\beta$ -HSD10) inhibitors.

We have designed, prepared and evaluated non-competitive ABAD (17 $\beta$ -HSD10) inhibitors of benzothiazolyl structural scaffold that were found effective in low micro molar range.<sup>2-3</sup> Further, the novel compounds were tested on the rate of oxygen consumption by mitochondria, activity of individual mitochondrial respiratory chain complexes, citrate synthase and monoamines oxidases with promising results.

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## Short communication MuTaLig 8

### Development of an Antibody Radiolabeled Drug Conjugate (ARDC) using $^{195\text{m}}\text{Pt}$ -Carboplatin for Theranostic Approach in Ovarian Cancer

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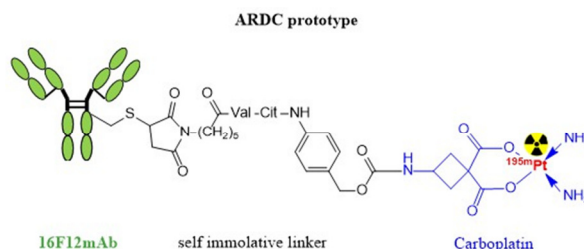
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In the past twenty years, advances in the field of antibody-drug conjugates (ADCs) have led to the commercialisation of two drugs, namely Kadcyla® for breast cancer and Adcetris® for Hodgkin lymphoma.<sup>1</sup> ADCs combine the high selectivity of monoclonal antibodies (mAbs) for their target with a highly potent cytotoxic payload. This allows minimisation of side effects of the drugs by specifically delivering the drug to cancer cells leaving healthy tissue unaffected. The vast majority of ADCs in clinical trials are aimed at oncological and haematological targets due to the large availability of mAbs against tumour markers. However, development of ADCs directed at application beyond oncology and haematology is on the rise.

Ovarian cancer is mostly diagnosed at an advanced stage and therefore survival rates are low. Treatment usually consists of surgery followed by chemotherapy based on carboplatin.<sup>2</sup> Radiotherapy is not used routinely for the treatment of ovarian cancer as it is difficult to localise the tumour due to its high dispersion. However, delivering the radionuclide specifically to the cancer cells opens up new opportunities for the treatment of ovarian cancer. In this context,  $^{195\text{m}}\text{Pt}$  is a particularly attractive radioisotope since it can be incorporated in drugs such as carboplatin.

The aim of this project is to develop antibody-radiolabeled drug conjugates (ARDC) which contain  $^{195\text{m}}\text{Pt}$ -labeled carboplatin to combine the DNA-damaging properties of  $^{195\text{m}}\text{Pt}$  and the DNA-alkylating properties of carboplatin with the highly selective delivery of the monoclonal antibody. Ovarian cancer cells are selectively targeted by 16F12 mAb, an antibody which is directed against human Müllerian Inhibiting Substance type II receptor (MISRII) and which was developed in our group.<sup>3</sup> This receptor is highly overexpressed in around 70% of ovarian cancers and thus represents a highly attractive target for treatment. Initial results (synthetic and biological) of this project will be presented.



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## Short communication MuTaLig 9

### Resveratrol analog induces hydrogen sulfide formation and vasorelaxation

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Hydrogen sulfide (H<sub>2</sub>S) is a gasotransmitter with important functions in neuromodulation, regulation of cardiovascular system and inflammation [1]. H<sub>2</sub>S inhibits reactive oxygen formation as well as causes vasorelaxation and protects myocardial cells from ischaemia. Previously we discovered that RVT (0.1 or 0.01 mM in penile tissue and aorta, respectively) induces H<sub>2</sub>S formation, relaxes the vascular tissues via H<sub>2</sub>S or decreases ROS formation through induction of H<sub>2</sub>S [2, 3]. Thus we now extended our research to find H<sub>2</sub>S-targeted drugs to new synthesized RVT-analogs ZMP17 and ZMP20 [4]

We measured the effect of RVT analog ZMP20 (10 μM) on H<sub>2</sub>S formation in the presence and absence of Cystathionine-gamma-lyase (CSE) inhibitor PAG (2 mM) by methylene blue assay in kidney and maximal relaxations to RVT and two RVT analogs ZMP17 and ZMP20 (10 mM) by DMT myograph in CD1 male mouse aorta. One Way Anova was used as statistical test.

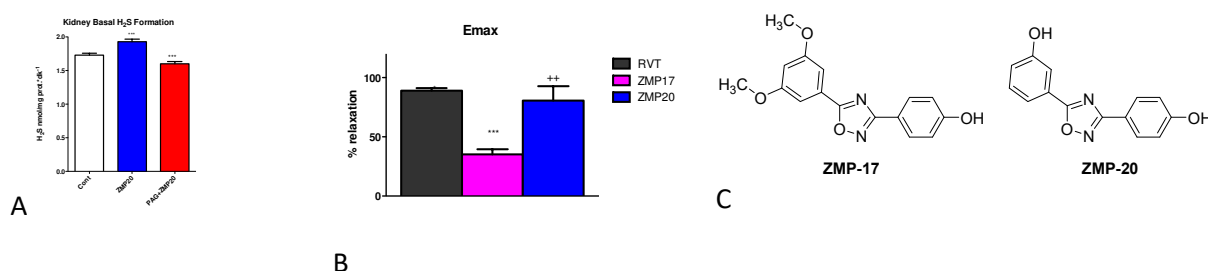


Figure 1: The effect of RVT analogs on A) H<sub>2</sub>S formation in murine kidney and B) vascular tonus in mouse aorta. C-Chemical structure of RVT analogs \*\*\*p<0.001 compared to control or RVT, +++p<0.01 compared to ZMP17, One Way Anova. n=3-5.

ZMP20 increased basal H<sub>2</sub>S formation in murine kidney tissue significantly (p<0.001, n=8). Inhibition of increased H<sub>2</sub>S formation by ZMP20 confirmed ZMP20 as an H<sub>2</sub>S-inducing drug (Figure 1, p<0.001, n=8.). RVT, ZMP17 and ZMP20 relaxed murine aorta (89.03 ± 2.122 vs 35.33 ± 4.214 and 80.53 ± 12.46). The maximal relaxation by RVT and ZMP20 was not different from each other but the relaxation induced by ZMP17 was significantly lower than that of RVT (p<0.001, n=4-5) and ZMP20 (p<0.001, n=3-5).

We concluded that RVT analog ZMP20 relaxes vascular tissues and induces H<sub>2</sub>S formation. ZMP20 may be beneficial in vascular or endothelial dysfunction. This study may be important to show the potential of RVT analogs as H<sub>2</sub>S-targeting drugs in cardiovascular diseases.

Acknowledgement: We thank TUBITAK for the grant #114s448 and the COST action CA15135.

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## Short communication MuTaLig 10

### Updates about the Chemotheca tool: status of implementation and use

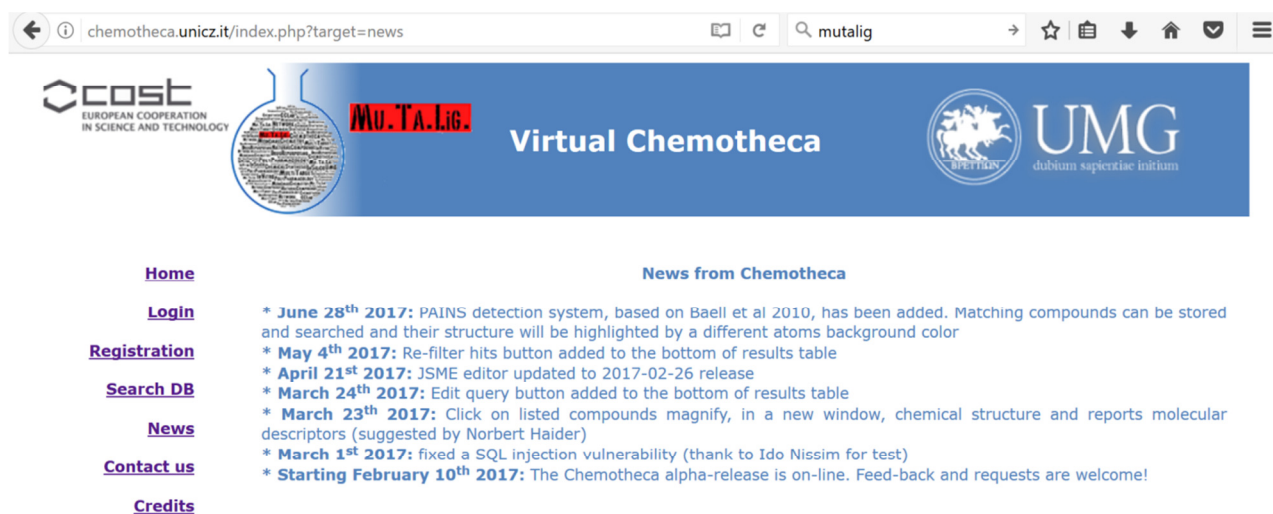
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The Chemotheca (figure 1) is the official virtual tool of COST Action CA15135. It has been designed to collect compounds from all MuTaLig participants with the purpose to create a dynamic molecular database useful for the identification of novel multi-targeting agents. The structure and the implementation of the Chemotheca is carried out at the Università Magna Græcia di Catanzaro and freely accessible connecting at the web address [chemotheca.unicz.it](http://chemotheca.unicz.it). During the 1<sup>st</sup> (Vienna, February 2017) and the 2<sup>nd</sup> (Siena, May 2017) training Schools of the MuTaLig COST Action the Chemotheca was presented to all participants. Since the goal of the COST Action is to expand the cooperation in a widely interconnected network among the pan-European parties, the Chemotheca was also presented at some non MuTaLig meetings. The participation of researchers is actually open to everyone, especially if they are young investigators.



**Figure 1:** screenshot of the Chemotheca tool.

In this communication we will briefly describe the technical characteristics of the Chemotheca in order to gain the participation of additional users, show the status of implemented compounds in the platform and announce the next steps of the virtual screening activities to be conducted on the basis of MuTaLig chemical database.

Moreover, a special initiative oriented to cover open journal expenses of manuscripts realized using the Chemotheca virtual tool among the MuTaLig community with 3 or more parties will be presented.



## Short communication MuTaLig 11

### Carbonic Anhydrase VA for the treatment of obesity: *in silico* identification of new inhibitors and prediction of anti-obesity side effects of FDA-approved drugs

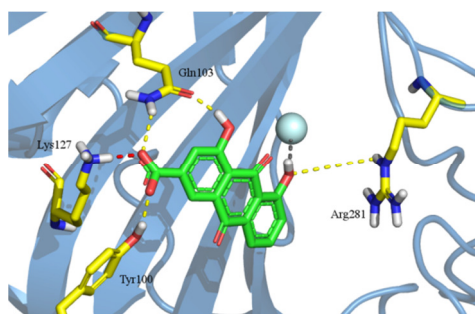
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Most drugs used for the management of obesity have serious cardiovascular or central nervous system side effects, which dramatically limit their usefulness. Recently, the inhibition of isoform VA of the carbonic anhydrases (CAs) has been proposed as a new anti-obesity strategy because of its function to decrease lipogenesis in adipocytes in cell culture<sup>1</sup>. Based on these data, we performed a structure-based virtual screening (SBVS) on the ZINC, FooDB and DrugBank databases of natural compounds and FDA-approved drugs *versus* the mitochondrial isoform VA of the CAs family. Starting from the X-Ray structure of the murine carbonic anhydrase deposited in the Protein Data Bank<sup>2</sup>, we built and refined the structure with the human isoform VA sequence by using UniProt database<sup>3</sup> and Prime homology modeling program<sup>4</sup>, respectively. After molecular dynamics simulations and the validation protocol, the Enrichment factor analysis was adopted to select the best model for the SBVS carried out by means of Glide<sup>5</sup> SP protocol. As a result, 15 *hit* compounds were selected and assayed on CAs VA function by means of enzymatic assay and, among them, an anthraquinone derivative (**Figure 1**) was identified as novel CAs VA inhibitor in the low nanomolar range.



**Figure 1:** 3D representation of the best pose of the anthraquinone derivative in complex with CAs VA.

In addition, among the selected drugs, such as Lenvatinib, Rufinamide and Fludarabine, we demonstrated a good correlation between the theoretical binding affinity towards the CAs and the experimental results, thus suggesting their implications in the unexplained weight loss, in agreement with the literature data<sup>6,7</sup>.

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## Short communication MuTaLig 12

### Pharmacophore models for identification of DNA gyrase and topoisomerase IV inhibitors and evaluation of their off-target binding

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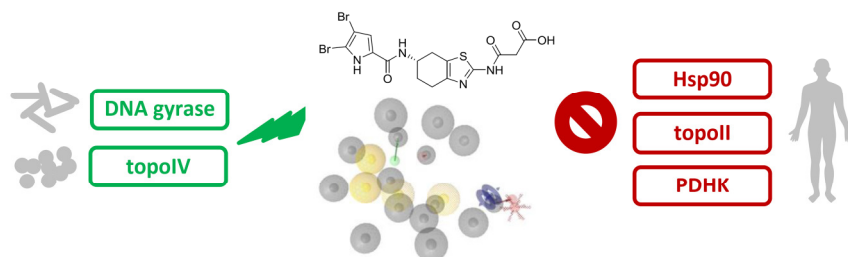
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The increasing emergence of pathogenic bacteria resistant to antibacterial drugs is a serious threat to global health and represents the continuous need for development of novel antibacterial drugs. Although ATP-competitive inhibitors of DNA gyrase subunit B (GyrB) and topoisomerase IV subunit B (ParE) are among the most studied classes of antibacterial agents, there is no representative in the antibacterial pipeline. Structural similarity of GyrB and ParE ATP binding sites enables the discovery of dual targeting inhibitors, which makes them attractive targets for antibacterial drug discovery. However, selectivity of GyrB and ParE inhibitors against closely related human ATP-binding enzymes should be evaluated early in the development to avoid off-target binding of advanced compounds in later stages.

Recently, we have prepared novel GyrB and ParE inhibitors with inhibitory activities in the low nanomolar range.<sup>1</sup> During the Short-Term Scientific Mission (MuTaLig COST Action), we developed on-target (GyrB and ParE) and off-target (e.g. topoisomerase II, Hsp90, pyruvate dehydrogenase kinase) 3D-chemical feature based pharmacophore models using LigandScout. Structure-based pharmacophore models were created based on x-ray derived enzyme-inhibitor complexes, while ligand-based models were created based on the known potent ligands. The models were validated and trained using sets of known active, inactive and decoy molecules and are important for hit finding, hit optimization support and activity profiling of previously prepared and novel GyrB and ParE inhibitors. Activity profiling of our GyrB and ParE inhibitors using these off-target pharmacophore models and LigandScout Expert KNIME predicted their selective on-target binding, which is also supported by some preliminary *in vitro* assays on Hsp90 and topoisomerase II.



**Figure 1.** Activity profiling of DNA gyrase and topoisomerase IV inhibitors.

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## Short communication MuTaLig 13

### Identification of G-Quadruplex DNA/RNA binders: structure-based virtual screening and biophysical characterization

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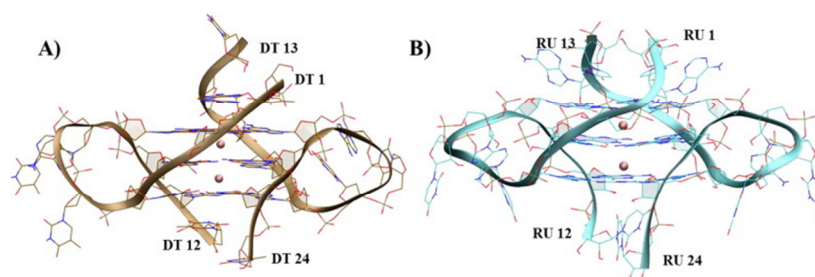
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Recent findings demonstrated that, in mammalian cells, telomere DNA (Tel) is transcribed into telomeric repeat-containing RNA (TERRA), which is involved in fundamental biological processes, thus representing a promising anticancer target<sup>1</sup>. On these grounds, the discovery of dual (as well as selective) Tel/TERRA G-quadruplex (G4) binders could represent an innovative strategy to enhance telomerase inhibition<sup>2</sup>. One of our last works was focused on these appealing targets. Initially, docking simulations of known Tel and TERRA active ligands were performed on the 3D coordinates of bimolecular G4 Tel DNA (Tel<sub>2</sub>) and TERRA (TERRA<sub>2</sub>) (**Figure 1**). Structure-based pharmacophore models were generated on the best complexes and employed for the virtual screening of around 257,000 natural compounds. Among them, 20 *hit* molecules were submitted to biophysical assays, which included circular dichroism and mass spectrometry at different K<sup>+</sup> concentrations. In particular, 3 *hits* were identified and characterized by biophysical assays. As a result, one of these compounds acts as dual Tel<sub>2</sub>/TERRA<sub>2</sub> G4-ligand, while the other two show preferential thermal stabilization for Tel<sub>2</sub> DNA. Our successful results pave the way to further lead optimization to achieve both increased selectivity and stabilizing effect against TERRA and Tel DNA G4s<sup>3</sup>.



**Figure 1:** 3D structures of the selected receptor models from the enrichment study. (A) Tel<sub>2</sub> and (B) TERRA<sub>2</sub>.

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## Short communication MuTaLig 14

### BitterPredict- a tool for predicting Bitterness of Drug candidate from its chemical structure

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Bitter taste is a significant factor in animal's choice of food. Animals avoid eating bitter food components, many of which are toxic. Nevertheless, it is known today that bitterness is not always noxious and that some of the bitter compounds have beneficial effects on health.

Drugs are often bitter, presenting compliance problems especially in children. Interestingly bitter taste receptors are also expressed in many extraoral tissues and emerge as novel targets for therapeutic indications such as asthma and infection.

Bitter compounds (gathered in the BitterDB<sup>1</sup> <http://bitterdb.agri.huji.ac.il/dbbitter.php>) dramatically vary in their structures. Therefore, identifying bitter molecules based on their chemical structures is a very challenging task.

Here we present a machine learning classifier, BitterPredict2, which predicts whether a molecule is bitter or not, based solely on its chemical structure. To this end we used: BitterDB as the positives set, non-bitter molecules that were gathered from literature as negative set, physicochemical and ADME/TOX descriptors for the molecules, and AdaBoost (decision tree based) algorithm.

BitterPredict correctly classifies over 80% of the compounds in the hold-out test set, and between 70% to 90% of the compounds in three independent external sets.

BitterPredict suggests that about 40% of random molecules, and a large portion (66%) of approved drugs, and of natural products (77%) are bitter.

The possible uses of the BitterPredict classifier range from basic questions related to evolution of taste and bitter taste receptors de-orphanization, to practical implications in drug and food industries. Predicting bitter compounds within the human metabolome may suggest unknown endogenous ligands of bitter taste receptors. Early flagging of potential bitterness of a drug candidate or key food ingredient can indicate the need for masking procedures and facilitate drug development.

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*EpiChemBio (CM1406) and MuTaLig (CA15135) COST actions joint meeting 2017 Porto (PT), Sept 22-24 2017*

## Poster communications

## Poster communication 1

### **Amoebicidal activity of $\alpha$ -bisabolol, the main sesquiterpene in chamomile (*Matricaria recutita* L.) essential oil against the trophozoite stage of *Acanthamoeba castellanii* Neff**

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#### **Abstract**

*Acanthamoeba* genus includes opportunistic pathogens which are distributed worldwide and are causative agents of a fatal encephalitis and severe keratitis in humans and other animals. Until present there are not fully effective therapeutic agents against this pathogen and thus the need to search for novel anti-amoebic compounds is urgent. Recently, essential oils of aromatic and medicinal plants have shown activity against *Acanthamoeba* strains. Therefore, this study was aimed to evaluate the activity of main component of chamomile essential oil (a sesquiterpene) namely  $\alpha$ -bisabolol against the *Acanthamoeba castellanii* Neff strain. After evaluation of the activity and toxicity of this molecule, IC<sub>50</sub> values of  $20.839 \pm 2.015$  for treated amoebae as well as low cytotoxicity levels in a murine macrophage cell line was observed. Moreover, in order to elucidate mechanism of action of this molecule, changes in chromatin condensation levels, permeability of the plasmatic membrane, the mitochondrial membrane potential and the ATP levels in the treated amoebic strains were checked. The obtained results revealed that  $\alpha$ -bisabolol was able to induce apoptosis, increase the permeability of the plasmatic membrane and decrease both mitochondrial and ATP levels in the treated amoebae. Therefore, and given the obtained results,  $\alpha$ -bisabolol could be used as a future therapeutic agent against *Acanthamoeba* infections.

## Poster communication 2

### **ZN/AU and ZN/AG complexes with SCHIFF Bases Ligand as new attractive antitumor agents**

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Schiff bases and their metal complexes attract the interest of scientific community because of their potential ability to react with different cellular/molecular targets and impressive biological activities including promising antitumor properties.

The aim of our study was to evaluate the influence of newly synthesized Zn/Au and Zn/Ag complexes with Schiff bases Salen, Salampy and Saldmen on viability and proliferation of a wide variety of model systems: i) cell lines established from the most common and socially important human neoplasia such as cancers of the breast and uterine cervix, non-small cell lung cancer and glioblastoma multiforme; ii) drug sensitive human squamous cell carcinoma A431 cells and resistant clones expressing ABC proteins ABCB1, ABCC1 or ABCG2 genes; iii) rat sarcoma (LSR-SF-SR) and chicken hepatoma (LSCC-SF-Mc29) cells that contain v-src or v-myc genes, respectively – the cellular analogues of these oncogenes are involved (when their normal regulation / function is disturbed) in pathogenesis of many cancers in humans and animals; iv) primary cultures obtained from transplantable myeloid tumor of Graffi in hamster and healthy lymphocytes and macrophages of the same laboratory animals; v) non-tumor human Lep-3 cells. The investigations were performed by short-term (3 – 72 h, with monolayer cultures) and long-term (30 - 40 days, with 3D cancer cell colonies) experiments using thiazolyl blue tetrazolium bromide test - MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, lactate dehydrogenase assay, double staining with acridine orange and propidium iodide, Comet assay, and colony forming method.

The results obtained revealed that applied at a concentration range of 0.01 - 20 µg/ml the examined compounds significantly reduced in a time- and concentration-dependent manner viability and proliferation of the treated cells. Zn/Au complexes were found to be more pronounced cytotoxic agents as compared to their Zn/Ag partners and in some cases are more effective than commercially available anticancer drug cisplatin.

### Poster communication 3

## Cabotegravir, a new Integrase inhibitor: drug stability evaluation

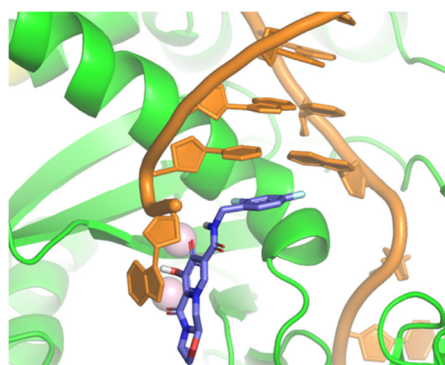
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Cabotegravir (CAB), an analogue of Dolutegravir (DTG), is a new HIV-1 Integrase inhibitor. CAB actually is in phase IIb development for HIV treatment as an oral tablet, and in phase IIb/III development for HIV prevention as long-acting injectable <sup>[1]</sup>. Starting from the crystal structure of the Prototype Foamy virus (PFV) intasome in complex with magnesium and DTG, deposited in Protein Data Bank (PDB) with 3S3M PDB code <sup>[2]</sup>, modelling studies were performed. Docking simulations were carried out by using Glide Standard Precision (SP) protocol by means of Glide v.7.2 <sup>[3]</sup>. OPLS\_2005 force field was used and 50 conformations were generated for each drug. Then, with the aim to evaluate the stability of CAB and DTG in complex with IN, 100 ns of Molecular Dynamics simulation (MDs) were performed by using Desmond package v.3.8 <sup>[4]</sup>. Our computational studies were in agreement with the experimental data and showed an higher theoretical binding affinity and a better stability of CAB *versus* IN with respect to DTG. Further experimental investigations are needed to confirm these results and the good activity of CAB in prevention and treatment of HIV.



**Figure 1:** 3D representation of CTG in IN catalytic binding site. The enzyme and the DNA are shown in green and orange cartoon, respectively. The drug is indicated as blue carbon sticks, while the magnesium ions are displayed as pink spheres.

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## Poster communication 4

### Supramolecular architectures for drug delivery systems

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The research at the interface of biomaterials, gene therapy, and drug delivery has identified several design parameters for the non-viral vectors to perform optimum delivery of biologically active material into cells. Huge progress has been made towards achieving gene delivery, though the design principles for the materials and non-viral vectors producing efficient delivery require continuous development and improvement.

This work summarizes some aspects concerning the design and preparation of efficient non-viral vectors for gene delivery, and antimicrobial drug system which inhibits biofilm formation.

Non-viral vector were obtained by approaching two different strategies: (i) preparation of core-like structures based on squalene derivative, fullerene C60,  $\beta$ -Cyclodextrine and cyclic siloxane conjugated with PEG and PEI<sup>1,2</sup> and (ii) Dynamic Constitutional Frameworks as non-viral vectors<sup>3</sup>. All developed vectors have the ability to complex plasmid DNA and thus deliver it to the cell.

The antimicrobial drugs are engineered dextran coated iron oxide nanoparticles, loaded with propiconazole, in order to test the hypothesis of a combined effect of the polymer and drug, both known for their antifungal activities.

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## Poster communication 5

### Targeted delivery of Mitochondriotropics derivatives: a new therapeutic approach for oxidative stress related diseases

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The mitochondrion is an important organelle for the genesis of ATP in which occurs the intracellular formation of the reactive oxygen species (ROS) and for that reason is particularly vulnerable to oxidative damage and consequently 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. In this context, it is believed that the modulation of mitochondrial dysfunctions by exogenous antioxidants can be a strategy to prevent or delay the deleterious oxidative effects present in aging and degenerative diseases, such as Alzheimer's disease. As AD is a multifactorial disease, the development of therapeutic agents that can reach two or more pharmacological targets involved in the neurodegenerative pathological cascade is attracting progressively more attention.

The main goal of this project is the development of innovative centrally active mitochondriotropic hydroxycinnamic derivatives with neuroprotective activity and able to cross the blood-brain barrier (BBB). The scaffold is a naturally occurring hydroxycinnamic acid (HCA) that has frequently been used as a model for the design and development of new antioxidants. However, despite exhibiting an interesting *in vitro* antioxidant activity its application in therapy was not successful. Failure of therapy is often associated with restraints related with physicochemical properties, particularly its low lipophilicity and bioavailability, which are not suitable for their biodistribution and penetration into the target site. Structural characterization of the newly synthesized compounds was carried out by NMR spectroscopy (<sup>1</sup>H, <sup>13</sup>C and DEPT) and electronic impact mass spectroscopy (MS/IE). Biological screening has included the assessment of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, *in vitro* antioxidant activity, *in vitro* blood-brain barrier permeation ability and the evaluation of compound's cytotoxicity in SH-SY5Y neuroblastoma cell lines. The results obtained so far will be presented in this communication.

This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2015, POCI-01-0145-FEDER-006980, NORTE-01-0145-FEDER-000028 and PTDC/DTP-FTO/2433/2014).

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## Poster communication 6

### Effect of catechols on the eradication of pre-formed *Escherichia coli* biofilms

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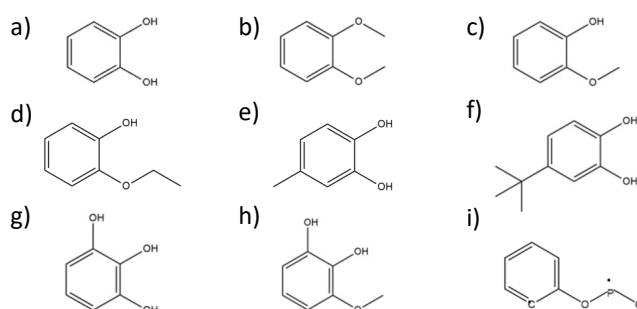
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Persistent bacterial infections and the increased resistance to multi-drugs are recognized as being related to biofilm formation and constitute a global concern in nowadays society. Furthermore, the effectiveness of current antibiotics is decreasing and few have been developed in the last decades. In this sense, studies need to be conducted in order to discover more efficient antibiofilm agents.<sup>1</sup> In this study, nine compounds with a catecholic moiety (catechol (CAT), veratrol (VER), guaiacol (GUA), 2-ethoxphenol (ETH), 4-methylcatechol (MEC), 4-tert-butylcatechol (TEB), pyrogallol (PYR), 3-methoxycatechol (MET), and o-phenylene-phosphochloridite (OPP)) (Figure 1) were investigated for their potential to eradicate pre-formed biofilms of *Escherichia coli*. Their action was assessed on biofilm mass and metabolic activity reductions, sessile cells' membrane integrity and culturability. ETH, MEC, TEB, PYR and OPP exhibited the best antibiofilm activities among the tested catechols, inducing biofilm removal and metabolic inactivation, sessile cells' membrane disruption and death. In general, an additive interaction of the tested catechols when combined with ciprofloxacin was attained in the control of *E. coli* biofilms. From a structure-activity relationship study it was possible to assess that an increase in the hydrocarbon side chain and lipophilicity using ETH and TEB as scaffolds would increase their activities. Moreover, the presence of hydroxyl groups in the selected catechols are suggested to be particularly related to their antibiofilm activity. All the selected catechols fulfil the Lipinski's "rule of five" requisites and thus are promising molecules to be used as scaffolds in the development of new formulations for therapeutic purposes.



**Figure 1:** Selected catechols. a) CAT; b) VER; c) GUA; d) ETH; e) MEC; f) TEB; g) PYR; h) MET; i) OPP.

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## Poster communication 7

### Novel chiral oxindole molecules for treating cancer

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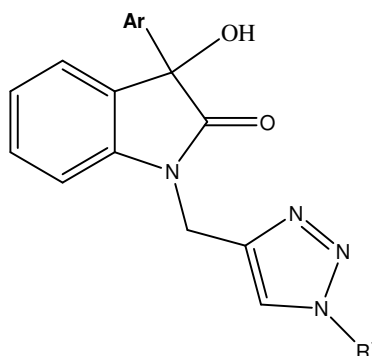
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Oxindoles and triazoles are very privileged frameworks in medicinal chemistry, and are thus ubiquitous in numerous medicines and natural products.<sup>1</sup> Molecules that contain both these privileged structures are highly desirable. We have developed a sequential catalytic route that involves the Sharpless-Meldal Cu-catalyzed alkyne-azide cycloaddition (CuAAC) followed by a catalytic arylation reaction to afford families of *N*-(1,2,3-triazolmethyl)-3-hydroxy-3-phenyloxindoles starting from cheap biomass derived isatin (Figure 1). We successfully obtained these compounds with good yields and good enantioselectivities.<sup>2</sup>



**Figure 1:** New catalytic route to chiral oxindole-triazole hybrids with anti-cancer properties

The compounds were then screened for anti-cancer activity, and showed very promising anti-proliferative activity for a variety of tumor cell lines. These results will be discussed in this presentation.

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## Poster communication 8

### **Cationic derivatives based on cinnamic acid as dual-target ligands for the treatment of neurodegenerative diseases**

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Aging-related neurodegenerative diseases, such as Alzheimer's disease (AD), are pathologies characterized by a progressive and irreversible neuronal death and a central nervous system dysfunction. As AD is a multifactorial disease, the development of therapeutic agents that can reach two or more pharmacological targets involved in the neurodegenerative pathological cascade is attracting progressively more attention. The generation of intracellular reactive species (ROS and RNS) by mitochondria dysfunction is thought to be one of the mechanisms that can lead to the progressive and irreversible neuronal death. In this context, it is believed that the modulation of mitochondrial function by exogenous antioxidants can be a strategy to prevent or delay the deleterious oxidative stress effects in neurodegenerative diseases.

The main goal of this project is the development of innovative centrally active mitochondriotropic hydroxycinnamic derivatives with neuroprotective activity and able to cross the blood-brain barrier (BBB). The scaffold is a naturally occurring hydroxycinnamic acid (HCA) that has frequently been used as a model for the design and development of new antioxidants. However, despite exhibiting an interesting in vitro antioxidant activity its application in therapy was not successful. Failure of therapy is often associated with restraints related with physicochemical properties, particularly its low lipophilicity, and bioavailability. Biological screening has included the assessment of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, in vitro antioxidant activity, in vitro blood-brain barrier permeation ability and the evaluation of compound's cytotoxicity in SH-SY5Y neuroblastoma cell lines. 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/00081/2013, POCI-01-0145-FEDER-006980, and NORTE-01-0145-FEDER-000028). F Cagide, S. Benfeito and C. Fernandes grants were also supported by FCT and FEDER/COMPETE funds.

## Poster communication 9

### Natural products in drug discovery: enhancing the Antioxidant profile of Hydroxycinnamic acids

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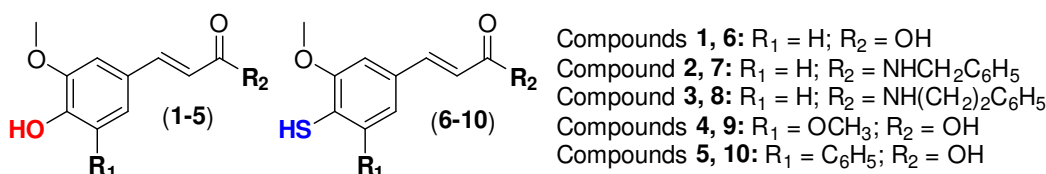
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Hydroxycinnamic acids (HCAs), an important class of polyphenolic compounds, have multiple activities with biomedical interest (e.g. antibacterial, antiviral, anti-inflammatory, neuroprotective) which have been ascribed to their antioxidant activity.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

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 FA (1) and related derivatives (2-10).

HCA-based antioxidants were successfully obtained. The antioxidant activity of the synthesized compounds was assessed by total antioxidant capacity assays, namely DPPH<sup>•</sup>, ABTS<sup>•+</sup> and galvinoxyl 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 oxidative damage inducers (6-hydroxydopamine, iron (III) and hydrogen peroxide). 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

### A green toolbox for the syntheses of N- and C-nucleosides

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N- and C-nucleosides are widely used in medicinal chemistry as therapeutic agents and/or biochemical probes. For example, the anti-cancer agent acadesin (AICAR) induces cell death through autophagy, an original mode of action allowing in some cases to circumvent tumor resistances to the conventional pro-apoptotic drugs. Various synthetic routes to N- and C- nucleosides have been disclosed in the recent literature. However, these strategies suffer usually from moderate yields, lack of stereo- and regio- selectivity, and use of hazardous reactants. In line with our previous studies, we report herein new synthetic processes using green conditions (including use of aqueous media and ultrasound activation), and non-hazardous reactants (eco-friendly Lewis acid catalysts), which are compatible with large scale syntheses for medicinal chemistry. The preliminary biological evaluation of several compounds highlights few "hits", to be optimized in the future.

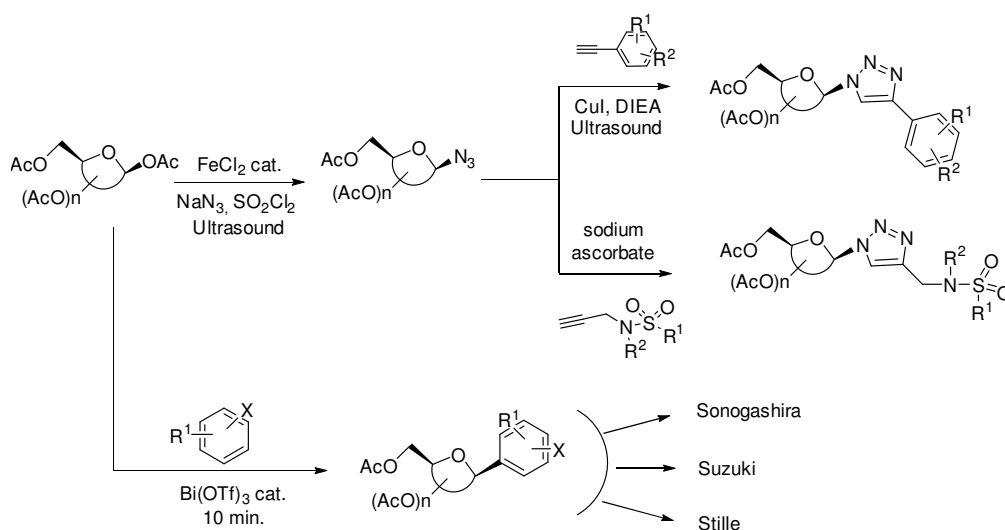


Figure 1

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## Poster communication 11

### Human Skin Fibroblasts from Parkinson's disease patients reveal metabolic alterations and oxidative stress

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Parkinson's Disease (PD) is a common neurodegenerative disorder, affecting more than 10 million people worldwide. Currently, PD has no cure and no early diagnostics method exists. Mitochondrial dysfunction seems to be an important component of the disease progression and it has been extensively demonstrated in PD models. To develop a personalized medicine approach to PD, one needs to identify cell proxies that can be used to test interventions aimed at improving mitochondrial function. Our hypothesis is that human fibroblasts may represent a minimally invasive tool to make an accurate diagnosis based on mitochondrial and/or metabolic alterations and to identify new strategies for treatment.

Human skin fibroblasts from PD patients and their respective matched controls were cultured in high-glucose Dulbecco's-Modified Eagle Medium (DMEM). A metabolic characterization of these cell lines was performed. Our results showed that ATP levels and glycolysis were decreased in human skin fibroblasts of PD patients, while oxidative stress was increased. However, no differences were found in oxygen consumption rate (OCR) or transcripts related with mitochondrial biogenesis, oxidative phosphorylation, mitochondrial dynamics, oxidative stress and autophagy. Metabolic viability, as measured by resorufin fluorescence was not different between groups. Our data demonstrate metabolic alterations in human skin fibroblasts from PD patients, although global mitochondrial function was not altered. Thus, human fibroblasts may represent a minimally invasive tool to study altered metabolism and interventions in PD.

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## Poster communication 12

### Effects of calorie restriction on methylation of Leptin Receptor Overlapping Transcript 1 in Breast Cancer Mouse model

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In today's world, two of the most widespread diseases are breast cancer and obesity. Both of them have high impact on patient's life. According to previous studies, it is known that there is a significant correlation between breast cancer and obesity, thus the preventive effects of calorie restriction (CR) on mammary tumor (MT) development has been suggested. However, the molecular mechanism(s) of how CR prevents or delays MT development is not clear. Beside to genetic aspects, epigenetic mechanisms play a pivotal role in the regulation of gene expression, therefore MT development can be controlled by epigenetic factors. Leptin is an adipokine which regulates energy balance and food intake. Recent studies have reported that leptin is necessary growth factor for normal mammary gland development and lactation, on the other hand it might also contribute to mammary tumorigenesis. In addition, higher leptin levels in obese people have been reported. Leptin Receptor Overlapping Transcript (LepROT1) decreases the response of leptin receptor for leptin by altering receptor-mediated cell signaling. In order to study the roles of *LepROT1* methylation on MT development under the effect of different CR types, MMTV-TGF $\alpha$  (+) mice which are prone to develop MT were divided into 3 groups: Ad-Libitum (AL), Chronic Calorie Restriction (CCR, 15 % CR application) and Intermittent Calorie Restriction (ICR, fed AL for three weeks, and the following week 60% restriction was applied from week 10 until week 82 of mouse age). Methylation of *LepROT1* in mammary fat pad was analyzed by pyrosequencing method. Methylation levels of *LepROT1* was increased with aging from wk 49/50 to wk 81/82 in AL, ICR-RF, and ICR-R groups whereas was decreased in CCR group. In addition, the methylation levels of *LepROT1* in CCR group was lower than the other groups at wk 81/82. Methylation of *LepROT1* may play important role in MT development since MT incidence rate was lower in CCR group compared to AL and ICR groups. (Supported by TUBITAK 114S429)

## Poster communication 13

### ***In silico* screening for the discovery of new inhibitors for the mutated protein BRAF-V600E**

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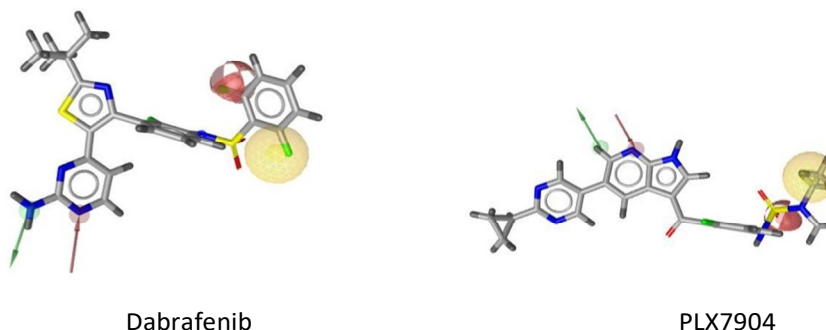
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The mitogen-activated protein kinase (MAPK) signaling pathway which affects cell proliferation, apoptosis, migration and differentiation has attracted the attention of anticancer research since abnormal activation of the pathway components is often identified in human cancers. BRAF belongs to the RAF family of serine/threonine protein kinases which are key regulators of the MAPK cascade. Activating BRAF mutations are harbored in certain cancers as in melanoma (50%), thyroid cancer (35-70%), colorectal cancer (5-20%), liver cancer (~14%) and ovarian cancer (~30%).<sup>1</sup>

BRAF-V600E is the most frequent mutation leading to multiple and uncontrolled amplification of downstream signal with tumorigenesis as a result<sup>2,3</sup>. Two selective BRAF-V600E inhibitors, Vemurafenib (Zelboraf) and Dabrafenib (Tafinlar), have been already approved for the treatment of unresectable and metastatic BRAF-mutated melanoma.

However, their efficacy is limited due to intrinsic resistance or the development of acquired resistance. Besides, in the context of wild-type BRAF cells, treatment with BRAF-V600E inhibitors leads to the paradoxical enhancement of MAPK signaling, resulting in enhancement of wt-tumour growth and adverse effects<sup>4</sup>. For that reason combined treatments are being tested with very good clinical outcomes<sup>5,7</sup>. A new generation of BRAF-V600E inhibitors (PLX7904 and PLX8394), being capable of overcoming the MAPK paradoxical activation, has been discovered recently and are currently in clinical investigations<sup>8</sup>.

We have conducted a virtual screening approach, utilizing structure-based pharmacophore modeling and *in silico* docking, towards the identification of novel, less prone to resistance, BRAF-V600E inhibitors. Pharmacophore model generation was based on the top-ranked features extracted from the respective models originated from the crystal complexes of BRAFV600E with the paradox breaker PLX7904 (pdb: 4xv1) and Dabrafenib (pdb: 4xv2), Fig. 1. We validated our models through ROC analysis by utilizing a library of 953 active and 62 inactive molecules recovered by ChEMBL database. ZINC database (12M compounds) was queried against the generated pharmacophore models and a number of 546853 molecules which reached or outdid our Pharmacophore fit score cut off were further filtered according to drug-like physicochemical properties. The set of "qualified" molecules are currently under investigation for their binding prediction, a process which is anticipated to provide the "eligible" molecules for biological evaluation.



**Figure 1:** Dabrafenib and PLX7904 fitted on the respective pharmacophore models.

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## Poster communication 14

### Synthesis of a Mitochondriotropic antioxidant based on Pegylation process

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Central nervous system (CNS) disorders, such as Alzheimer's disease, are among the most emergent, disabling and yet non-curable diseases of the 21<sup>st</sup> century. The complex etiology of the CNS disorders as well as the cascade of events leading to the neurodegenerative process is not completely understood, but it is known that the unbalance between reactive oxygen and nitrogen species (ROS/RNS) and endogenous antioxidant led to oxidative stress, followed by neuronal damage processes and eventually cell death.

Since that the most part of the ROS/RNS are produced in mitochondria, mitochondriotropic antioxidants can be a successful approach as they are able to penetrate in the phospholipid bilayer of mitochondria's membrane and have the capacity of accumulation in the negatively charged compartments of the mitochondrial matrix.<sup>1</sup>

However, some issues related with the crossing of drug through blood–brain barrier (BBB) are still a challenge for the researchers. To overcome these drawbacks, efforts have been focused to improve drug delivery using carriers with lipid or polymeric origin. Drug delivery carriers can help to reduce the number of doses required and the degradation of the drugs, and to improve the solubility and bioavailability. Polyethylene glycol (PEG), a well-known hydrophilic polymer, had been reported as a tool to improve the stealth properties of several nanoplateforms, increasing their abilities to cross BBB.<sup>2</sup>

The aim of the work is to synthesize polymer-phenolic conjugated systems that can cross BBB, maintaining the antioxidant properties of phenolic moiety. The polymer-conjugated drug ability to trigger biological responses and the toxicity profile will be carried out by suitable in vitro studies as well as its permeability in brain endothelial cells. The acquirement of bioavailability and pharmacokinetic properties of the best systems will be also performed.

This work was supported by the Foundation for Science and Technology (FCT), Portugal. C. Fernandes (SFRH/BD/98519/2013) grants are supported by FCT.

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## Poster communication 15

### Quinoline-based chalcogenazoles: synthesis and biological evaluation

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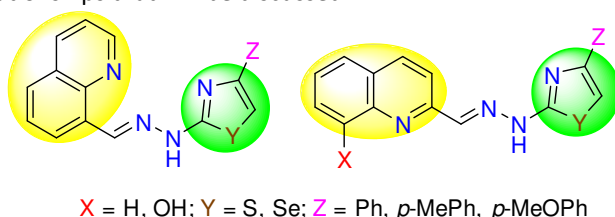
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Under the umbrella of MuTaLig, we have started a collaboration to obtain potential multi-target compounds by the combination of two privileged scaffolds: quinoline and 1,3-thiazole. On the one hand, quinoline and its analogs have recently been examined for their modes of function in the inhibition of tyrosine kinases, proteasome, tubulin polymerization and DNA repair [1]. On the other hand, 1,3-thiazole ring plays vital roles in many antineoplastic, anti-HIV, antifungal, antiparasitic, anti-inflammatory, and antiulcer agents [2]. In order to expand the biological scope of our compounds, we envisioned selenium analogues of 1,3-thiazoles, which have shown better biological and antioxidant activities [3].

We have prepared a small and focused library of 18 compounds (Figure 1). All the derivatives were analyzed for their antiproliferative activity, antioxidant potency, and for glycosidase and acetylcholinesterase inhibition. The results provided structure-activity relationships that will be discussed.



**Figure 1:** General structures of investigated quinoline-based chalcogenazoles.

The results allowed us to identify lead compounds that will be further studied for GEP analysis in order to determine the affected pathways and establish the scope of our compounds as potential anticancer and anti-Alzheimer's drugs.

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## Poster communication 16

## Multiple target compound design incorporating toxicity and anti-targets

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Compounds can interact with multiple partners. One way of using this in designing chemical probes or drugs is through master key compounds that interact with several protein partners in a desired way. Natural products can also have multi-target interactions, both for useful bioactivities, or for non-desired interactions.<sup>1</sup> One way of early flagging of non-desired interactions is through the use of anti-targets, a case of off-targets.<sup>2,3,4</sup> It is shown in this work, how design of N-myristoyl transferase inhibitors can be achieved for compounds that can have potential to inhibit several parasites, *Leishmania*, *Aspergillus*, and *Plasmodium* at once, while avoiding interaction with human proteins such as metabolism and efflux or clearing proteins. Additionally, compounds for oncological treatment have been initially screened. A cocktail of substances presents another challenge for multiple interactions. This may be exploited as an advantage, such as the use of ritonavir in addition to other antivirals for HIV treatment.<sup>5</sup> Closely related natural products can also affect several pathways. Also presented is how compounds can be modeled in order to assess their toxicology, in this case, for the US EPA challenge, CoMPARA. In addition, the QsarDB<sup>6</sup> will be presented, where compounds and models have been represented digitally with published data for a variety of endpoints, including ecotoxicity, human health, and other critical properties of compounds.

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## Poster communication 17

### Microencapsulation as a strategy to improve the ADMET properties of bioactive caffeic acid derivatives

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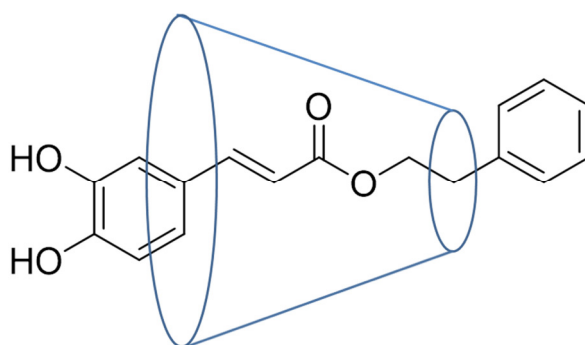
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Caffeic acid phenethyl ester (CAPE) is a bioactive polyphenolic compound obtained from propolis extract. Although it has a broad therapeutic potential its bioavailability is limited, due to reduced solubility, and stability in both plasma and systemic circulation. Cyclodextrins are cyclic oligosaccharides arising from the degradation of starch, that have been proved to be promising excipients for the formulation of active ingredients. As a result of its molecular shape and structure, cyclodextrins exhibit the unique ability to trap a guest molecule inside its cavity and act as a molecular container. In fact, CDs have the ability to modify the guest molecule's characteristics, enhancing their solubility, stabilizing them against external agents, controlling volatility and sublimation properties and the release of the encapsulated compounds.

In this presentation, we report the inclusion complexation behavior and binding ability of CAPE with hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD). The supramolecular interaction was examined through UV and FTIR spectroscopy, DSC,  $^1\text{H}$  NMR ROESY. The CAPE/HP- $\beta$ -CD inclusion complex was formed at a molar ratio of 1:1 (Figure 1) and its stability constant was determined to be  $2911.6 \text{ M}^{-1}$  in water and  $2866.2 \text{ M}^{-1}$  at physiological pH. The aqueous solubility increased notably probing that HP- $\beta$ -CD encapsulation can be potentially a useful strategy to improve the ADMET properties of CAPE.



**Figure 1:** The proposed structure of the 1:1 HP- $\beta$ -CD:CAPE inclusion complex.

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## Poster communication 18

### Synthesis, characterization and microbiological activity of a new PAMAM Metallo dendrimer

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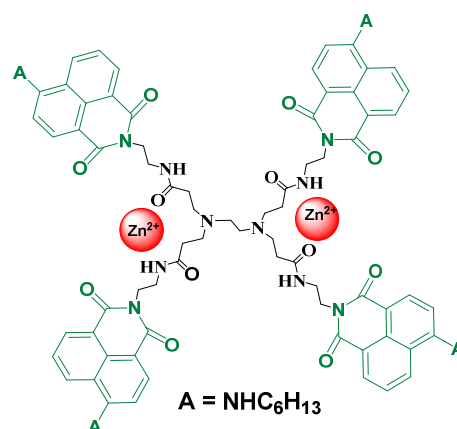
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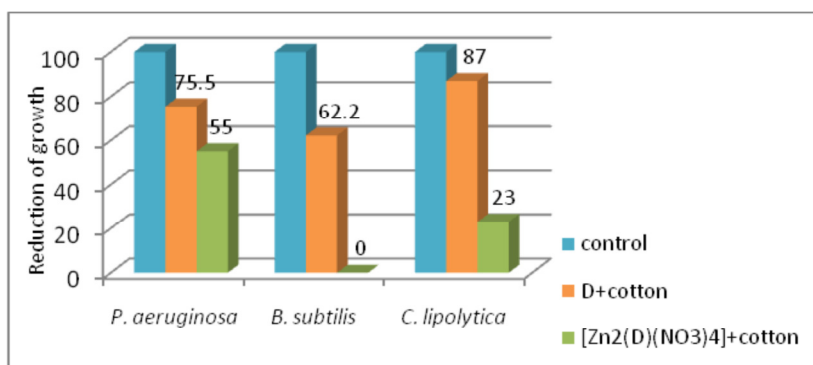
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In this study we present the results from the synthesis and spectral characterization of a new PAMAM metallo dendrimer modified with four 4-hexylamino-1,8-naphthalimide. The chemical structure of metallo dendrimer has been analysed with FTIR, Electronic and NMR spectroscopy (Figure 1). The antimicrobial activity of PAMAM dendrimer D and its Zn complex  $[Zn_2(D)(NO_3)_4]$  was investigated in agar medium and in meat-peptone broth against several pathogenic bacteria and yeasts. The *in vitro* antimicrobial screening showed good inhibition activity of the compounds, which is better expressed against the test Gram-positive bacteria and yeasts. The antimicrobial efficacy of cotton fabrics treated with both dendrimers was evaluated. Their anti-adhesive and biofilm inhibiting potential were analysed by SEM. Deposition of the metallo dendrimer  $[Zn_2(D)(NO_3)_4]$  on the textile fabric prevents the formation of a biofilm, and its effect is stronger than those of the free of metal ions ligand. This is a good indicator of using this dendrimer to produce antibacterial textiles (Figure 2).



**Figure 1:** Chemical structure of metallo dendrimer  $[Zn_2(D)(NO_3)_4]$



**Figure 2.** Reduction of the growth of model bacteria *P. aeruginosa* and *B. subtilis* and the yeast *C. lipolytica* by untreated and treated cotton fabric.

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## Poster communication 19

**Derivatives of *TERT*-AMYLPHENOXYALKYL as histamine H<sub>3</sub> RECEPTOR ligands and MONOAMINE OXIDASE B inhibitors**

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Histamine H<sub>3</sub> receptors (H<sub>3</sub>Rs) are predominantly found in the region associated with cognition, sleep, wakefulness or homeostatic regulation. Their blockade increase the release of histamine itself and other neurotransmitters such as acetylcholine (ACh), dopamine (DA), noradrenaline (NA) or serotonin (5-HT) [1]. Histamine H<sub>3</sub>R blocking compounds (antagonists/inverse agonists) are interesting target in the search for new drugs, for the potential treatment of Alzheimer's Disease, ADHD, allergic rhinitis, narcolepsy, obesity or schizophrenia (2).

Monoamine oxidases (MAO) are enzymes responsible for the metabolism of the monoamine neurotransmitters (e.g. serotonin, dopamine), and a variety of xenobiotic amines (3). So far two MAO isoforms have been identified: MAO A and MAO B. MAO B, in the central nervous system, is involved not only in regulating the level of neurotransmitters (such as dopamine), but also in the processes leading to damage of nerve cells in the cholinergic neurons.

In this work we synthesized analogs of our previously described potent histamine H<sub>3</sub> receptor ligand - 1-[3-(4-*tert*-amyl-phenoxy)propyl]piperidine (**DL77**) (4). Firstly, compounds were evaluated on their binding properties at the human H<sub>3</sub>R. Secondly, an inhibition of the human recombinant MAO B was checked. The target compounds showed human H<sub>3</sub>R affinities in nanomolar range (K<sub>i</sub> < 800 nM). Some of them we also able to inhibit MAO B. The most potent compounds showed MAO B inhibitory activity of 20 nM.

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## Poster communication 20

### Design of new Peptide-Based Vectors for Blood-Brain Barrier targeting and CNS drug delivery

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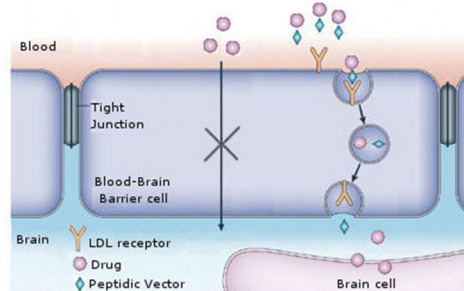
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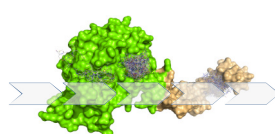
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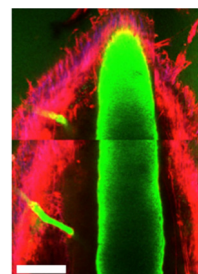
Drug delivery to the brain is hindered by the presence of the blood–brain barrier (BBB). To accomplish the task of nutrient transport, the brain endothelium is endowed with a diverse collection of molecular transport systems. Such systems include receptor-mediated transcytosis (RMT), which employs the vesicular trafficking machinery of the endothelium to transport substrates across the BBB. This system can also be used to shuttle a wide range of therapeutics into the brain as a non-invasive manner. Over the last decade, there have been significant developments in the area of RMT-based brain drug delivery.<sup>1</sup> In this field, members of the low-density lipoprotein receptor (LDLR) family are relevant as drug transport systems.<sup>2</sup> Consequently, the main goal of this project is dedicated to the development of new peptide-based ligands of LDLR as potential new BBB-vectors. The initial screening of a phage-display library directed to LDLR led to the identification of hits such as a cyclic 15-mer peptide with high *in vitro* affinity. From this compound, a chemical optimisation was carried out through Ala-scan, D-scan, truncation, non-natural residues insertion, configuration changes... This led to the identification of a new lead peptide, a cyclic 8-mer with one non-natural residue. To evaluate its ability to act as a BBB-vector, it was coupled with a model opioid peptide known to be unable to cross the BBB on its own. Brain uptake as well as *in vitro* and *in vivo* biological effects of such bivalent conjugate were then measured giving promising results. These first results were then assessed by biphotonic microscopy imagery.<sup>3</sup>



Ac-[cMPRLRGc]-NH<sub>2</sub>



CNS-targeting via  
LDL receptor



CNS delivery

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## Poster communication 21

### Cytotoxicity and Fluorescent studies of Molecular Logic Gates for pH and pE

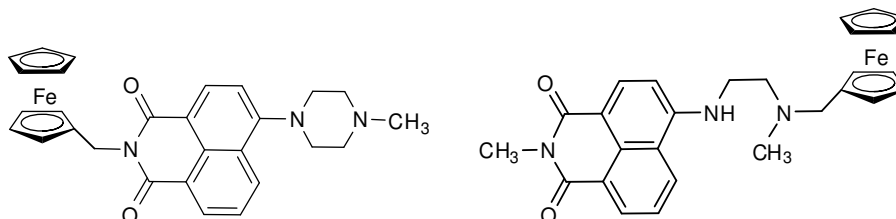
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From the cross-fertilization of fluorescent pH indicators with fluorescent redox switches, our group has invented a new class of molecular logic gates known as 'Pourbaix sensors'.<sup>1,2</sup> These molecular sensors are named in honor of Marcel Pourbaix, who long ago appreciated the relationship between the redox potential (pE) and proton concentration (pH) of metal ion species in aqueous solution.<sup>3</sup> Typically associated with corrosion processes, oxidisability and acidity are also important parameters in medicine, as for example in the successful development of the ferrocene antimalarial candidate, ferroquine.<sup>4</sup> Our recent efforts have been on the development of 'Pourbaix sensors' with a naphthalimide scaffold and a tertiary amine receptor for protons and a ferrocenyl moiety for oxidants. The fluorescent switching characteristics are controlled by photoinduced electron transfer (PET) and internal charge transfer (ICT) mechanisms according to an AND logic algorithm.<sup>5,6</sup> In this presentation, particular attention will be given to the synthesis, cytotoxicity and fluorescent cell imaging studies of such molecules as shown in Figure 1.



**Figure 1:** Examples of 'Pourbaix sensors' as potential cytotoxic and fluorescence imaging agents.

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## Poster communication 22

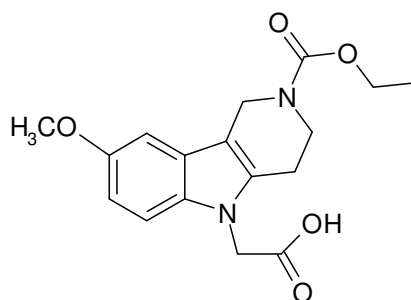
### Tricyclic derivatives of Indole-1-acetic acid as Aldose Reductase Inhibitors

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Aldose reductase (ALR2) plays an important role in the pathology of chronic diabetic complications and disorders related to inflammation. Therefore, searching for efficient and safe ALR2 inhibitors is in the center of interest of many academic and commercial research units. Derivatives of indole-1-acetic acid represent one of the most prospective compounds with many additional positive biological effects. Among them, compounds with tricyclic structure showed the best inhibition activity and selectivity towards aldehyde reductase. The group of triazino-indole derivatives is represented by 5-carboxy-3-mercapto-1,2,4-triazino-[5,6-b]indole (cemtirestat) inhibiting ALR2 in submicromolar range, while the study of carboxymethylated tetrahydropyridoindoles brought several novel inhibitors with  $IC_{50}$  up to the value 12.6 nM represented by 2-(2-(ethoxycarbonyl)-8-methoxy-3,4-dihydro-1H-pyrido[4,3-b]indole-5(2H)-yl) acetic acid (Fig.1) Both compounds were tested on organ level and in vivo experiments. Profitable inhibition properties were confirmed also for isolated rat lens and red blood cells. In vivo experiment of streptozotocin induced diabetes on rats revealed an early ability to inhibit sorbitol accumulation in sciatic nerve. Concurrently, we started to build up the systematic database of compounds with single indole and pyridoindole structure, whose properties have been studied by a virtual screening method to find optimal pharmacokinetic and pharmacodynamics properties.



**Figure 1:** Structure of 2-(2-(ethoxycarbonyl)-8-methoxy-3,4-dihydro-1H-pyrido[4,3-b]indole-5(2H)-yl) acetic acid

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**Acknowledgement:** This work was supported by VEGA 2/0041/15 and VEGA 2/0033/14. We also thank the Slovak Research and Development Agency under the contract No. APVV-15-0455 and SAS – TÜBİTAK Joint Project No. JRP 2015/7 for funding.



## Poster communication 23

### Effect of the ligand spacers on the antioxidant activity of Manganese(III)-Schiff base complexes

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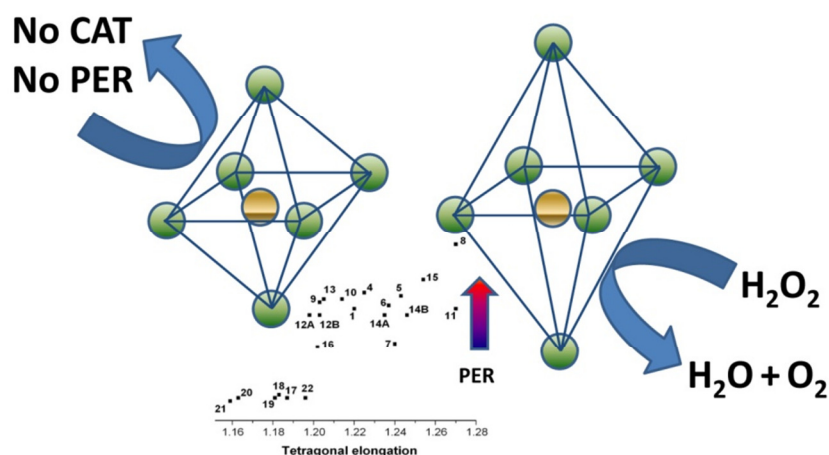
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Oxidative stress causes uncontrolled oxidation of organic molecules with secondary alteration of their structure and biological functions, leading to the progressive deterioration and the collapse of organs and systems in the living organisms. The superoxide dismutase, catalase and glutathione peroxidase enzymes are part of the defense mechanisms against the reactive oxygen species generated by oxidative stress. We are seeking for artificial models for these redox enzymes using metallocompounds that can be built by means of supramolecular self-assembly strategies induced by appropriate ligand design.



**Figure 1:** Correlation between the factor of tetragonal elongation and peroxidase activity for the tested compounds.

In this communication we report the study as peroxidase, catalase and SOD mimics of a series of manganese complexes using Schiff bases with different spacers between the imine groups. The length of this spacer arises as a key factor to enhance the peroxidase and catalase activity of the biomimetic models. SOD activity does not follow the same pattern. Moreover this work includes discussions about the structural factors that determine the best antioxidant behavior, the limitations of the ABTS method to evaluate antioxidants and also the cytotoxicity tests for these artificial models against human erythrocytes.



## Poster communication 24

### Development of new NIK inhibitors for the potentiation of anti-cancer immunotherapy

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Immunotherapy by inhibition of checkpoints is a major advance in cancer treatment, but response rates remain low (10 to 57% depending on the type of cancer and the treatments used). Studies focusing on new molecules used in immunotherapies, such as anti-PD1 and anti-PDL1, permitted to evidence the key role of gamma-interferon (IFN- $\gamma$ ) in the response to such immunotherapies.<sup>1, 2</sup>

We have shown that the inhibition of the non-canonical pathway of NF- $\kappa$ B and its upstream kinase, NIK, restores a senescence program in melanoma cells by decreasing the transcription of EZH2, a major oncogene.<sup>3</sup> On the basis of these results, we initiated a medicinal chemistry program aiming at uncovering new pharmacological inhibitors of NIK (NIKi). We eventually gained access to a family of compounds that are as effective as the NF $\kappa$ B2-directed siRNA. In fact, both decreased EZH2 transcription while concomitantly restoring a senescence program. Notably, our small-molecule inhibitors do not cause any side effects or toxicity in non-cancer cells. Inhibition of the NF $\kappa$ B2 non-canonical pathway, either by siRNA or via NIKi, in melanoma, colon and lung cancer cells results in the production of IFN- $\gamma$  by these cells, *in vitro* and in syngeneic mouse models. This modified secretome induces a strong attraction of the immune cells, M1 polarization of the macrophages and the activation of T lymphocytes and dendritic cells. Moreover, NIKi reduces the size of subcutaneous tumors without causing apparent toxicity, *in vivo*. When an anti-PD1 treatment is associated, a clear synergistic effect was observed. While separately the anti-PD1 and NIKi only slow the tumor progression, their association induces a major reduction in the size of the tumors that could even result in complete regression in several cases.

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## Poster communication 25

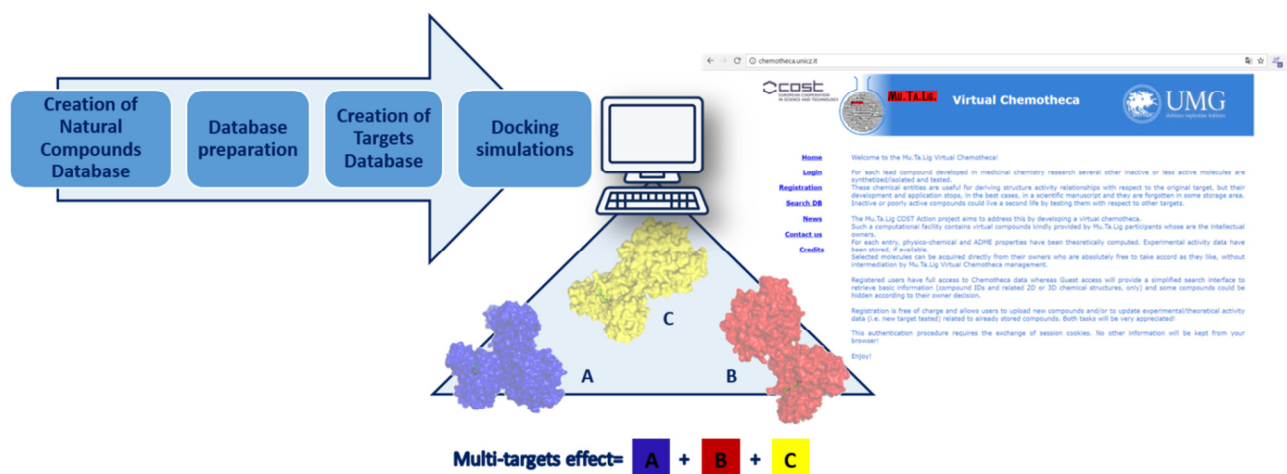
### Chemoinformatic database creation and *in silico* hit-identification of Multi-Targeting bioactive compounds

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Designing drugs that can simultaneously interact with multiple targets is a promising approach for treating complicated diseases. 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<sup>1</sup>. The first step of the work-flow is the creation of chemoinformatic databases (compounds extracted from plants and fungal species), available online on the Chemothea web site<sup>2</sup>, useful to perform *in silico* studies with the aim to identify potential active compounds *versus* different targets of medicinal chemistry interest and to evaluate if some compounds may present polypharmacological activity for the design of new multi-target agents.



**Figure 1:** Computational approaches applied in polypharmacology and virtual screening techniques.

Furthermore, virtual drug screening method help identify sources of off-target drug effects and investigate their potential to cause adverse or desirable side effects (Figure 1).

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## Poster communication 26

### Docking-based drug repurposing to predict new small molecules for Alzheimer's disease: a multi-target approach

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Alzheimer's disease (AD), the most common cause of dementia, is a chronic disorder characterized by a progressive decline in cognitive function. The currently approved drugs are only palliative and symptomatic. Several overlapping physiopathology mechanisms have been proposed for AD, such as: cholinergic deficit, extracellular deposition of  $\beta$ -amyloid plaques, formation of intracellular neurofibrillary tangles, neuroinflammation, oxidative stress, and excitotoxicity.<sup>1</sup> Taken into consideration such a multifactorial scenario, our research activity was focused on the identification of new multi-target-directed ligands by means of molecular modelling techniques.

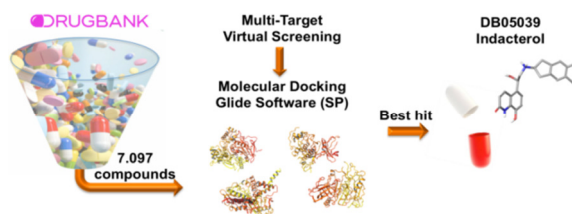


Figure 1: Virtual screening workflow.

In particular, 7,097 compounds retrieved from DrugBank database, were virtually screened by Glide software toward crystal structures of AChE, GSK-3 $\beta$ , BACE-1 and MAO-B. In fact, repurposing of already approved drugs has the advantages to capitalize on previous investments and to escape two of the main reasons for failure in drug approval, i.e. poor toxicological and pharmacokinetic profile. Our results indicated indacaterol, nowadays approved for the treatment of chronic obstructive pulmonary disease (COPD), as potential inhibitor of all investigated targets. Currently, the selected compound is submitted to biological assays.

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## Poster communication 27

### 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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## Poster communication 28

### Hydroxybenzoic acid-based mitochondriotropic antioxidants as cholinesterase inhibitors

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Neurodegenerative diseases (NDs) are a heterogeneous group of disorders related with genetic and environmental factors, among others, having an enormous impact in human health. Alzheimer's disease (AD) is the most prevalent type of ND and dementia. AD is currently associated to cell-altered oxidative stress status, a process that is related with a failure in the antioxidant protective system and/or an increment in reactive species production/accumulation. As a result, this process causes the destabilization of cellular membranes, damage of blood-brain barrier, disintegration of DNA and ultimately, neuronal death.

Accordingly, neuroprotective agents with an extended therapeutic window are urgently needed. Therefore, the aim of this project has been focused on the design and synthesis of innovative lipophilic hybrid mitochondriotropic antioxidants using benzoic acid as a scaffold. Mitochondriotropic compounds are characterized to have the ability to rapidly cross phospholipid bilayers without requiring a transporter and, consequently, to accumulate within the mitochondria.

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<sup>+</sup>). After synthesis, purification and structural identification the in vitro antioxidant profile was evaluated using ABTS and DPPH assays. In addition, they have also been screened toward cholinesterase enzymes (AChE and BChE) as they are a key AD targets. Their cytotoxic and neuroprotective profile was evaluated in human neuroblastoma (SH-SY5Y) and in human hepatocellular carcinoma (HepG2) cell lines. The results obtained so far will be presented in this communication.

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

## Poster communication 29

### Antiproliferative activity of unprecedented thiocyanate silver complexes

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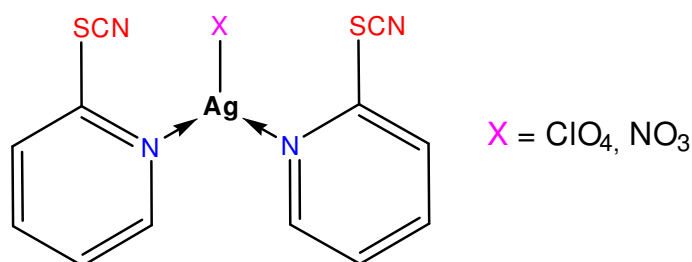
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Silver complexes exhibit significant in vitro antiproliferative activities. They arise as an alternative to cisplatin, a drug in clinical use for cancer chemotherapy. An additional advantage of silver compounds in the development of new metallothepapeutic drugs, is their low toxicity to humans. Although the mechanism of their activity is not well established, it has been suggested that silver(I) compounds exert antiproliferative effects by interacting with DNA and through binding to thiol groups of the proteins by inducing apoptosis [1].



**Figure 1:** Structures of antiproliferative thiocyanate silver(I) complexes

We have synthesized two unprecedented silver complexes with a readily accessible pyridine-based monodentate ligand. The structures of the complexes were determined by single crystal X-ray crystallography. In a preliminary screening against human solid tumor cell lines, both complexes showed GI<sub>50</sub> values in the range 2-4 μM. This data together with mechanistic studies will be presented.

#### Acknowledgements

N.R.F. thanks MuTaLig COST Action CA15135 for an STSM.

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## Poster communication 30

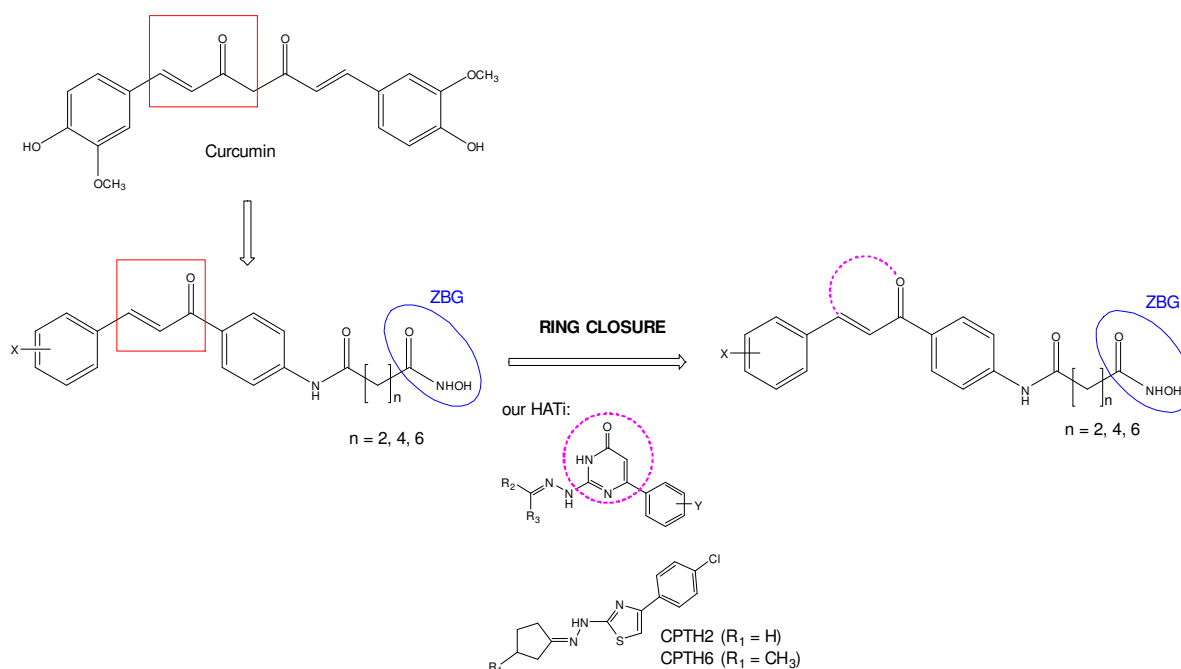
### New chalcone derivatives as Hat/Hdac modulators

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Histone acetyltransferases (HAT) and histone deacetylases (HDAC) are key enzymes involved in the complex machinery which regulates the genome accessibility through decondensation/condensation of the chromatin structure. The abnormal activity of these enzymes has been associated with several diseases, therefore underling the importance of the development of new modulators. Based on the modulator activity of Curcumin<sup>1</sup>, our group has designed and synthesised novel chalcone derivatives (**Figure 1**).



**Figure 1:** Structural modifications leading to new HAT/HDAC modulators.

The new compounds have a carbonic chain of variable length functionalised with an hydroxamic moiety (ZBG), with the aim to confer the ability to coordinate the catalytic zinc of HDACs.

On the basis of the structure of HAT inhibitors that we previously synthesised,<sup>2,3</sup> we additionally performed different ring closures on the chalcone moiety in order to assay their activity as HAT and HDAC modulators.

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## Poster communication 31

### Playing with Pharmacophore Modeling to design an Epigenetic modulator

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Enhancer of Zest Homolog 2 (EZH2) is the catalytic subunit of Polycomb repressive complex 2 (PRC2), which has been shown to play a critical role in the epigenetic regulation of cancer. The trimethylation of lysine 27 in Histone 3, catalyzed by EZH2 is a process that facilitates chromatin compaction and gene silencing.<sup>1</sup> It is known that some worse prognosis cancer types, as lymphoma, are related with overexpression and gain of function mutations of the enzymatic complex PRC2.<sup>2</sup> Thus, the inhibition of PRC2, namely the inhibition of EZH2, appears as an opportunity to anticancer therapy.

A computational-aided drug design campaign (CADD) intended to generate design models for hit finding and lead optimization research was implemented to identify new EZH2 inhibitors. Thus, we created 3D-chemical feature based pharmacophore models, including ligand and structure based models using LigandScout Advanced 4.1.4 software.<sup>3</sup> The most relevant structure-based pharmacophore (SBP) was experimentally derived from X-ray data of protein ligand complex PDB-ID 5LS6. This model consisted of 8 features (hydrophobic and hydrogen-bond acceptor-HBA) as well as 16 excluded volumes. The HBA features are important for “anchoring” the molecule in the preferential conformation for interaction with the EZH2 active site. Some relevant Ligand-based pharmacophoric models (LBP) were also created from a set of active ligands reported in the literature. The most robust model, that retrieves most of the active known inhibitors, presents 14 features (Hydrophobic, HBA, hydrogen-bond donor and aromatics). Merged and shared-feature pharmacophore models were created to explore structure-activity relationships (SAR) and improve the prediction capacity of the models. The performance of all the models were tested using virtual screening libraries (created using LigandScout) of known inhibitors of EZH2, inactive and decoy molecules. The most predictive models were optimized by systematic modification on the features of the models. These models will be used for hit finding campaigns and lead optimization medicinal chemistry decision support. Furthermore, the CADD methods provided a foundation for our SAR hypotheses that are crucial to design more accurate and reliable 3D-pharmacophores as the project evolves.

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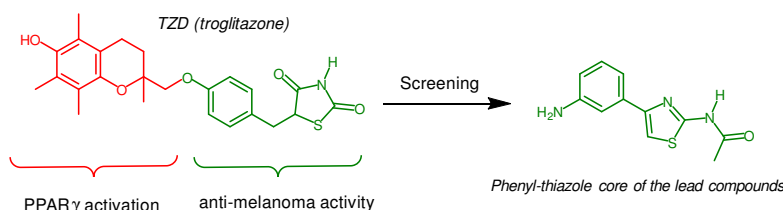
### ER Stress Inducers Targeting Resistant Cancers

Cyril Ronco,<sup>a</sup> Antoine Millet,<sup>a</sup> Magali Plaisant,<sup>b</sup> Michael Cerezo,<sup>b</sup> Emilie Jaune,<sup>b</sup> Stéphane Rocchi,<sup>b</sup> Rachid Benhida<sup>a</sup>

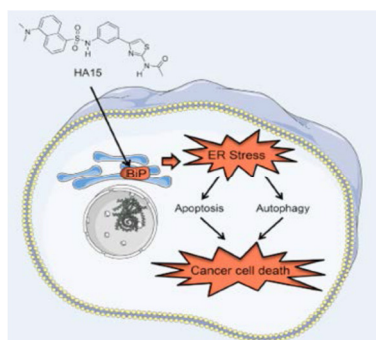
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<sup>b</sup> Université Côte d'Azur, UNS, Centre Méditerranéen de Médecine Moléculaire (C3M) – INSERM U1065

Despite dramatic improvement in its care over the last two decades, the treatment of resistant forms of cancer is still an unmet challenge. Thus, efficient treatments, especially relying on innovative modes of action are needed to overcome these resistance phenomena. We have recently discovered that thiazolidinediones (TZD), which are known PPAR $\gamma$  receptor ligands used in the treatment of type 2 diabetes display also non-negligible **death induction of melanoma cell lines** by apoptosis. We have modified the TZD structure in order to improve the efficacy towards neoplastic cells. Therefore, we have discovered **a new class of anticancer molecules** displaying a phenyl-thiazole core, active on many sensitive and resistant cancer cells.<sup>1,2</sup>



Extensive iterative structure–activity and structure-pharmacological properties relationships allowed defining two optimized lead compounds active in vivo. Meanwhile, the study of the mode of action of one of those named **HA15** was extensively studied by transcriptomics, SiRNA, western blots, cytotoxic activity measurements and microscopy methods to disclose an original mode of action: the induction of **Endoplasmic Reticulum (ER) stress** and a selective death of cancer cells by concomitant apoptosis and autophagy.<sup>3</sup>



An original molecular target was then identified by proteomics, immunoprecipitation, fluorescence co-localization, ITC, DSC and ATPase activity inhibition techniques: the molecular ER-chaperones **GRP78 / BiP**. These enzymes play a pivotal role in the so-called Unfolded Protein Response, an ER-stress adaptation mechanism necessary for the survival of cancer cells.

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<sup>2</sup> Ronco, C.; Millet, A.; Plaisant, M.; Rocchi, S.; Benhida, R. et al. *Bioorg. Med. Chem. Lett.* **2017**, 27, 2192-2196.

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## Poster communication 33

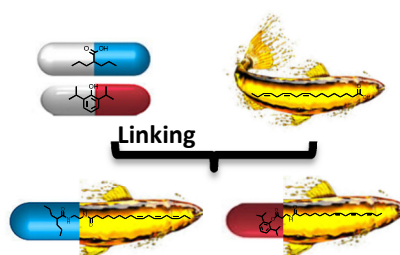
### Design, synthesis and characterization of polyunsaturated fatty acid conjugates: towards a potential epigenetic polypharmacology

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Polyunsaturated fatty acids (PUFAs) are essential dietary lipids, which regulate a variety of cellular functions.<sup>1</sup> In particular, PUFAs are well-known epigenetic regulators that activate sirtuin deacetylases.<sup>2</sup> Valproic acid<sup>3</sup> and propofol<sup>4</sup> are two neurological drugs with an intrinsic epigenetic activity. The first one acts as an inhibitor of HDAC, whereas the second one normalizes the alterations induced on histone H3 by toxic insults. The therapeutic interest for a potential epigenetic polypharmacological approach is rapidly increasing.<sup>5</sup> On this basis, we have designed and synthesised PUFAs-conjugates with a therapeutic potential towards neurological diseases. To this end, the structures of alpha-linolenic acid and valproic acid/propofol are joined covalently by ester or amide linkers.<sup>6</sup> (Figure 1)



**Figure 1:** Linking approach between valproic acid/propofol with alpha-linolenic acid.

These PUFAs-conjugates should be inactive in the circulation, so that safety may be improved compared to the individual starting drugs.<sup>5</sup> Once delivered inside the target cells, they potentially become substrate of specific intracellular enzymes that release the PUFA and valproic acid/propofol, which, in turn, should be able to simultaneously modulate multiple epigenetic targets.

The PUFAs-conjugates have been preliminary tested in hydrolysis and epigenetic assay. The epigenetic effects of PUFAs-conjugates have been evaluated analyzing histone H3 lysine acetylation in a model of neuronal cells in vitro.

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## Poster communication 34

### Evaluation of active natural products as bacterial efflux pump inhibitors

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Natural products emerge as important molecules with a wide range biological activities. To name a few, antibacterial, antifungal, antimalarial, anti-HIV, antitumoral, vasorelaxant, and neuroprotective activities are among the many different properties they may possess.<sup>1</sup> As they impose their activities, natural products commonly bind to multiple targets.<sup>2</sup> This is a desirable property since available evidence suggests that single-target drugs do not always induce the desired effect because of the activation of compensatory pathways.<sup>3</sup> The current work investigates the binding of structurally similar and/or biologically active natural products to multi-drug efflux pumps to block their efflux function along with their activities. To this end, the bioactive compounds alkaloids (roemerine, boldine and bulbocapnine) and essential oils (carvacrol and thymol) were selected as inhibitor candidates. Roemerine, boldine and bulbocapnine are aporphine type alkaloids with very similar structures. Previously we have reported only roemerine as an active antimicrobial against *Bacillus subtilis*. On the other hand the essential oils, both carvacrol and thymol were reported to possess antimicrobial activities targeting the cellular membranes. For the transport tests, berberine, a compound extracted from a variety of herbs, was used as the substrate since it is known to be effluxed through these pumps. Based on the minimum inhibitory concentration (MIC) of berberine for berberine, *B. subtilis* cells were treated with combinations of berberine (75 µg/mL, below the MIC value) and one of the selected potential pump inhibitors. The concentrations of the potential inhibitors were selected as not to affect the growth of *B. subtilis*. Growth was monitored by measuring OD<sub>600</sub>. Our results have shown that the 75 µg/mL berberine + 25 µg/mL roemerine and 75 µg/mL berberine + 50 µg/mL thymol combinations resulted in death of *B. subtilis* cells. On the other hand, the boldine, bulbocapnine or carvacrol showed no effect on cells when combined with 75 µg/mL berberine. These findings strongly support the hypothesis that the bioactive compounds roemerine and thymol also act as pump inhibitors in *B. subtilis* however which class of multi-drug efflux pumps is inhibited should be further investigated.

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## Poster communication 35

### Multi-targeted ChEI-copper chelating molecules as neuroprotective agents

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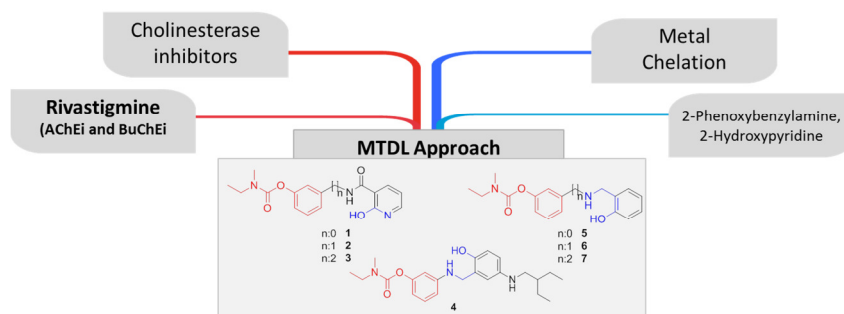
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Alzheimer's disease (AD) is one of the leading causes of death from mental and behavioral disorders in Europe. AD is a progressive and irreversible brain disorder that slowly destroys memory and thinking skills. Some of the multiple pathogenic mechanisms contributing to the pathological hallmarks of AD are the  $\beta$ -amyloid generation, neurofibrillary tangles (NFTs) formation, neuro-inflammation and aberrant mitochondrial activity. Several therapeutic approaches have been proposed even if they show a quite limited effectiveness and are mostly provided as palliative therapy. The multiple biological profile of AD prompted us to design and synthesize a series of multi-targeted molecules by the combination of cholinesterase inhibitors and antioxidant agents<sup>1,2</sup>. Growing evidence show that the homeostasis of metals such as copper, zinc, and iron is significantly altered in AD brain. Consequently, pursuing our medicinal chemistry campaign in the search for new multi-targeted drugs for AD therapy, we combined rivastigmine skeleton with metal chelating moieties such as 2-hydroxy-pyridine (**1-3**) or 2-hydroxy-benzylamine nucleus (**4-7**). *In vitro* studies showed that the new molecules are both inhibitors of cholinesterases and self  $\beta$ -amyloid<sub>1-42</sub> aggregation. Moreover, the new hybrids were able to chelate metals and showed both antioxidant and neuroprotective activities in HT22 cells against glutamate-induced neuronal death. In summary, the new compounds represent valuable hit molecules for further studies as neuroprotective agents



**Figure 1:** Multitarget ChEI-chelating copper molecules

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## Poster communication 36

### Dual targeting of Adenosine ( $A_1/A_{2A}$ ) receptors and Phosphodiesterase 10A by 2-amino-pyrimidin-3-carbonitriles

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Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by gradual motor function impairment caused by the progressive loss of dopamine (DA) producing neurons.<sup>1</sup> The classical approaches in the treatment of this disease have long been associated with undesirable effects.<sup>1,2</sup> As a result, non-DA based approaches have emerged as alternative approaches.<sup>2</sup> The modulation of adenosine receptors has recently arisen as an appealing approach to treat PD.<sup>3</sup> It has been reported that antagonism of  $A_1$  and  $A_{2A}$  receptors inhibits PD in a synergistic manner.<sup>3</sup> Thus, blockage of  $A_1$  receptor facilitates DA release,<sup>3</sup> while  $A_{2A}$  antagonists enhances postsynaptic responses to DA.<sup>3</sup> Moreover, the high expression of phosphodiesterase 10A (PDE10A) in the striatal medium spiny neurons, and its role in motor control, support that PDE10 inhibitors may be attractive pharmacological agents in PD.<sup>4</sup> In the frame of a project aimed at the development of multitarget ligands for complex pathologies, and starting from our in-house collection of potent and selective adenosine receptor antagonists, we herein document the synthesis, structure-activity relationship and molecular modeling of 2-aminopyrimidin-3-carbonitriles (Figure 1) as dual  $A_{2A}$ -PDE10A and  $A_1$  - PDE10A ligands.



**Figure 1:** General structure of the library and pharmacological profile of identified ligands

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## Poster communication 37

### Synthesis and antimicrobial activity of new water-soluble quaternary ammonium Anthraquinone

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In this study we report the synthesis and characterization of a new cationic water-soluble anthraquinone derivative (AN) having quaternary amino group (Figure 1). The antibacterial activity against various Gram-positive and Gram-negative bacteria and strains has been investigated *in vitro*. The results obtained suggest that the newly synthesized compound is effective in treating the relevant pathogens and is suitable in designing new effective antimicrobial preparations. (Figure 2).

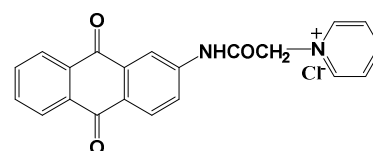


Figure 1: Chemical structure of AN

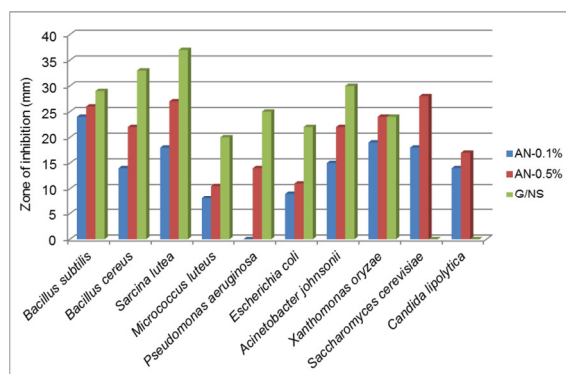


Figure 2. Zones of inhibition of the growth of the studied bacteria and yeasts

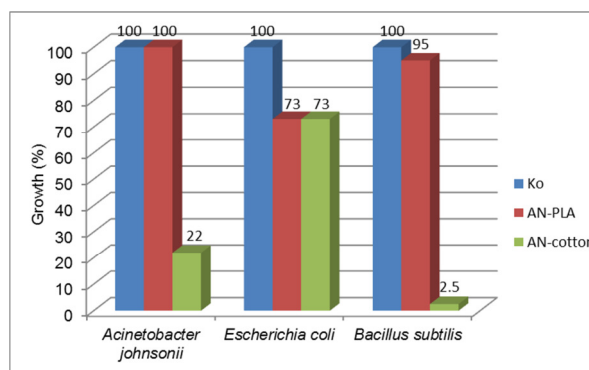


Figure 3. Effect of AN-PLA film and AN-cotton specimens on the growth of pathogens tested in meat-peptone broth

The incorporation of the AN into thin polylactide film and on the cotton fabric surface has been investigated. The results of *in vitro* antimicrobial tests of the obtained AN-polylactide film and AN-cotton samples are shown in Figure 3. After 24h of treatment, the AN-PLA film specimen reduced the growth of *E. coli* and *B. subtilis* by 27% and 5%, respectively, and no growth inhibition of *A. johnsonii* was established. The AN-cotton specimen caused a significant decrease in the growth of *A. johnsonii* and *B. subtilis* by 78% and 97.5%, respectively, while for *E. coli* this decrease was much lower (27%).

The gradual release of AN from both polymer matrices into water solution has been also investigated. It was demonstrated that the compound released exhibited a prolonged and good antibacterial activity.

#### Acknowledgements

The authors acknowledge Grant № KOCT, 03-2017, Fund "Scientific Research", Ministry of Education and Science of Bulgaria.



## Poster communication 38

### Cholic Acid Conjugation as a Tool for Enhancing Intracellular Delivery of Linker-Extended Constructs<sup>†</sup>

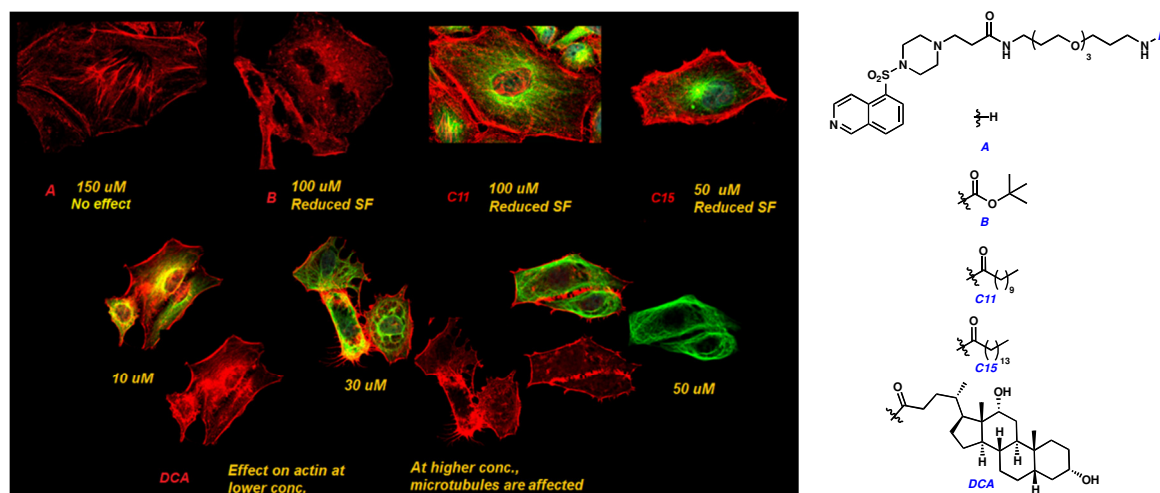
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Chemical tagging of biologically active entities is instrumental to the research at the interface of chemistry, biology and biomedicine. Such tools deliver reliable ways to study existing, discover novel and molecularly engineer new variants of mechanisms of action for a plethora of chemical probes, including small molecules, gene products (primarily, proteins) and conjugates thereof.

A major challenge when designing probes that bear additional functionality is their decreased cellular uptake, which is essential for probes aimed at intracellular targets. The extension of a given probe by a polyethyleneglycol (PEG) or other linker often results in strongly diminished or no intrinsic biological activity. To address this challenge, we report that direct conjugation to cholic acid derivatives serves as a useful tool in overcoming decreased biological activity of the linker-extended constructs. Moreover, we systematically explore structure–activity relationships of cholic acid conjugation using cell biological assessment of the constructs targeting three unrelated targets to demonstrate the potential utility of this approach.



**Figure 1:** Effect of lipid anchoring on the potency of linker-extended constructs of multi-target ROCK inhibitor HA-100.

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## Poster communication 39

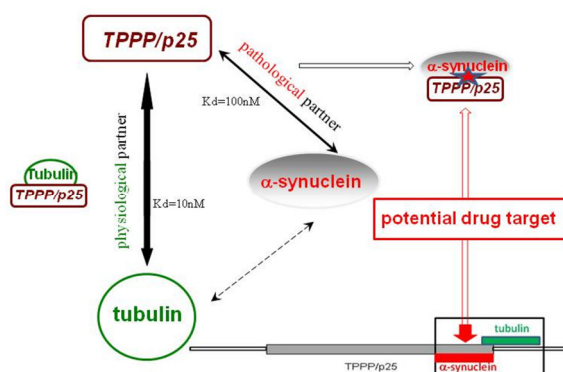
### Double life of the Multifunctional Disordered TPPP/p25: *pathological function*

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The highly dynamic cytoskeletal microtubule system undergoes constant and rapid reorganization that plays crucial roles in a number of cellular events such cell division, differentiation and pathological inclusion formation. Our research focuses on a recently discovered brain specific protein denoted Tubulin Polymerization Promoting Protein (TPPP/p25), that we identified as a disordered Microtubule Associated Protein with neomorphic moonlighting characteristics meaning it displays both physiological and pathological functions. In normal brain, TPPP/p25 is expressed predominantly in oligodendrocytes and plays crucial role in the differentiation of the progenitor cells by stabilizing the microtubule network during projection growth. However, this multifunctional protein is co-enriched and co-localized with  $\alpha$ -synuclein (SYN), the hallmark of synucleinopathies such as Parkinson's disease (PD).

Recently we have reported a new innovative strategy for validation of specific anti-PD drug targets<sup>1</sup>, based upon the targeting of the interface of the pathological TPPP/p25-SYN complex, which prevents pathological complex formation, without affecting the physiological TPPP/p25-tubulin interaction. Using various deletion mutants of the partner proteins for the in vitro and cellular studies including the Bimolecular Fluorescence Complementation coupled fluorescent microscopy; we have identified the binding segments of the complexes involved in the pathological complex formation.



**Figure 1.** Targeting the interface of the  $\alpha$ -SYN-TPPP/p25 complex without affecting its physiological functions.

This binding segment is localized within the highly flexible CORE part of the disordered protein straddled by the unstructured N- and C-termini. We have shown that this CORE segment associates with neither the tubulin nor the intracellular microtubule network while it does bind to  $\alpha$ -synuclein. Since the pathological complex is formed by the association of two unstructured proteins with chameleon feature the question was whether this segment is responsible exclusively for the formation of the pathological complex. A number of deletion mutants were produced by recombinant techniques used for in vitro and in vivo binding studies that revealed the high conformational plasticity of TPPP/p25 which can ensure exceptional functional resilience; the lack of the identified binding segments could be replaced by other segments<sup>2</sup>. All these data revealed that although targeting chameleon proteins is a challenging task, nevertheless, the validation of a drug target can be achieved by identifying the interface of complexes of the partner proteins existing at the given pathological conditions.

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### ***In vitro* characterization of potential biological applications of MITOK<sub>3</sub>, A NOVEL Mitochondriotropic Menadione derivative**

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Vitamin K<sub>3</sub>, containing the 2-methyl-1,4 naphthoquinone structure lacking the side chain, has potent cytotoxic activity mainly resulted from its quinone redox-cycling with production of reactive oxygen species (ROS). Despite, increased ROS generation is considered as a key mechanism in the induction of cancer cell death, it may not be suitable to efficiently kill cancer cells. Therefore, combinations of ROS-generating agents with other molecules targeting important features of the cancer cell physiology can be beneficial. As mitochondrial dysfunction has been implicated in a plethora of human diseases, including cancer, mitochondria are important and attractive targets for drug discovery.

We developed a new mitochondrial-targeted agent based on menadione (MitoK<sub>3</sub>) by conjugating a TPP cation and an aliphatic lipophilic spacer to the C3 position of the naphthoquinone ring that seems to sensitize mitochondria to oxidative stress-induced events. The chemical modification performed in menadione structure allowed specifically accumulation in mitochondrial matrix but also interfered with its redox properties and consequent biological outcome. MitoK<sub>3</sub> sensitize mitochondrial by inducing a dysfunction on mitochondrial bioenergetic apparatus, with subsequent loss of mitochondrial ATP production. Thus, it might be a suitable strategy to increase the cytotoxicity of anticancer agents, such as DOX. The combined strategy with oxidative stress-inducing agents, such as DOX and MitoK<sub>3</sub> was shown to be effective even below its death-induction threshold, and triggered apoptotic cell death evident by caspase 3/9 activities, probably through mitochondrial destabilization or by interference with mitochondrial redox processes.

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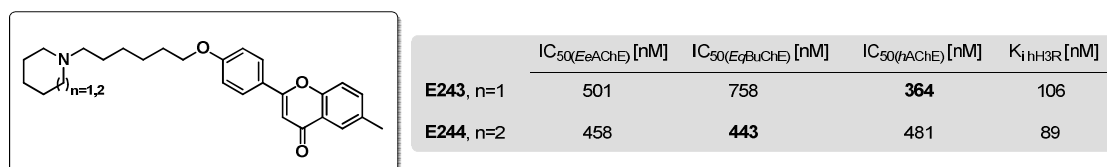
### MULTIFUNCTIONAL LIGANDS TARGETING HISTAMINE H<sub>3</sub> RECEPTOR AND CHOLINESTERASES

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Nowadays, Alzheimer's disease (AD) is one of the most common form of dementia, affecting about 30 million people. Current available treatment is capable of only bringing the symptoms down, but cannot modify the progression of the disease [1]. The loss of cognitive functions in patients with AD is related mainly to progressive decrease of cholinergic transmission. One of the recent therapeutic strategies in AD is the use of the multifunctional ligands acting in a synergistic manner on different targets. Cholinesterases (AChE, BuChE) are responsible for acetylcholine (ACh) hydrolysis. Additionally, it was found that histamine H<sub>3</sub> receptor activation is responsible for inhibiting the release of ACh in the central nervous system. With these considerations in mind, a great tool to improve the cholinergic neurotransmission can be a combination of histamine H<sub>3</sub> receptor antagonism with AChE and/or BuChE inhibition. The aim of our study was to search for the multi-target-directed-ligands as potential anti-AD agents. In order to achieve this goal a library of 186 non-imidazole ligands of H<sub>3</sub> receptors was explored in docking-based virtual screening to evaluate its potential inhibitory activity towards AChE. Twenty-six compounds were selected for investigation of the cholinesterase inhibitory profiles using the method established by Ellman et al. [2] Most of the selected hits exhibited cholinesterase inhibitory activity with IC<sub>50</sub> values in submicromolar range. The greatest achievement was recognition of two potent AChE and BuChE inhibitors, E243 and E244 with high H<sub>3</sub> receptor affinities (Figure 1).



**Figure 1:** The structure of the lead compounds.

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 Prefix PLE = plenary lecture EpiBioChem  
 Prefix PLM = plenary lecture MuTaLig  
 Prefix SCC = short communication common topics  
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