Fourth WG Meeting CA15135

BOOK OF THE ABSTRACTS

COST ACTION CA15135

Final status of WG activities within the MuTaLig COST Action

Ege University, Faculty of Pharmacy, Bornova, İzmir, Pearl of the Aegean March 5th - 6th 2020

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INTRODUCTION

The MuTaLig COST Action concludes the cycle of 12 meetings and training schools as planned in the Memorandum of Understanding document before starting its pan-European activities. The diffusion of this event has been done, as usual, by means of the contact e-mail list, that recently has increased up to more 800 units. The four-year journey allowed to create a consistent network that includes 33 nations, most of them belonging to the Inclusiveness Target Countries. Izmir arrives at the end of the COST Action after the Annual Meetings done in Lugano, Porto, La Valletta and Catanzaro, the Training Schools in Vienna, Siena, Hamburg and Lugano and the WG meeting of Budapest, Tenerife and Paris. It can be asserted that all Europe was involved from North to South and from East to West. This is exactly within the cooperation spirit stated by the COST Association.

The WG meeting in Izmir is the perfect occasion to talk about the final status of the MuTaLig activities with the aim to complete the Final Assessment report and to look forward to other implications. Along with the 28 selected oral communications 34 poster communications will complete the scientific section. The last part of the WG meeting will be dedicated to projects already in progress within the MuTaLig community, including the CIG application and other plans.

As Chair of this COST Action, I want to express my gratitude especially to the local organizer and LOS (Prof. Gunay Yetik-Anacak, MC member for Turkey) for her enthusiastic work, to her local team, to the Grant Holder from University of Porto (Prof. Fernanda Borges and Dr. Susana Maria Ventura da Costa) and to the COST Association (Dr. Lucia Forzi, Science Officer and Dr. Svetlana Voinova, Administrative Officer) for their efforts in the meeting organization. A special thank is also due to the young investigator Dr. Antonio Lupia (Net4Science academic spinoff hosted at Università "Magna Græcia" di Catanzaro, Italy) for the support in the organization of this abstract book.

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

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ACKNOWLEDGMENTS

The MuTaLig COST Action acknowledges the following institutions and sponsors for the support given to the Organizing Committee of the Fourth WG meeting.

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- Dr Gülşah Bayraktar

PhD students

- Elif Alan
- Merve Saylam
- Nazlıcan Belen
- Emine Nur Özbek

Sponsors and Institutions

PROGRAM

Thursday, March 5th 2020

8.30 Registration

9.00 **Introduction to the MuTaLig COST Action 4th WG meeting**
Necdet BUDAK (Rector) – Ege University (Turkey)
Ayfer YALÇIN (Dean of Faculty of Pharmacy) – Ege University (Turkey)
Stefano ALCARO (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)
Günay YETİK-ANACAK (MC member Turkey and local organizer) – Ege University (Turkey)

Body percussion dance show with mortar and pestle
"A multi-targeting pharmacist: Galen from Aegean land"

Reports of the WG leaders
Moderator: Fernanda BORGES – (Vice-Chair and MC member Portugal) University of Porto (Portugal)

Danijel KIKELJ – (WG1 leader) University of Ljubljana (Slovenia)
Eugenio GAUDIO – (WG2 leader) Oncology Research Institute, Bellinzona (Switzerland)
Sharon BRYANT – (WG3 leader) Inte:Ligand GmbH, Vienna (Austria)
Hanoch SENDEROWITZ – (WG4 leader) Bar-Ilan University, Ramat-Gan (Israel)

10.30 *Coffee break*

Session I "Multi-targeting agents against cancer and infections"
Moderator: David C. MAGRI – (MC member Malta) University of Malta (Malta)

11.00 OC1 **Toward Multitarget DNA Gyrase Inhibitors Possessing Antibacterial Activity**
Danijel KIKELJ – (WG1 leader) University of Ljubljana (Slovenia)

11.15 OC2 **Repositioning of [1,6] Naphthyridines as Antiviral Agents on HSV-1**
Alessandra MONTALBANO – University of Palermo (Italy)

11.30 OC3 **Indolocarbazole maleimides with dual inhibition of kinases and topoisomerase I**
Florence McCARTHY – (MC member Ireland) University College, Cork (Ireland)

11.45 OC4 **Epigenetic Multitargeting in Cancer: Design, Synthesis And Biological Evaluation of Novel Inhibitors**
Filipa RAMİLO-GOMES – University of Lisbon, Lisbon (Portugal)

12.00 OC5 **The effect of New Synthesized Resveratrol Derivate H1 on angiogenesis**
Emine Nur ÖZBEK – Ege University, İzmir (Turkey)

12.15 OC6 **A Comparative Study of The Antiangiogenic Activity of Hydroxytyrosyl Alkyl Ethers as Multitargeted Bioactive Compounds**
Miguel Ángel MEDINA – University of Málaga, Málaga (Spain)

12.30 OC7 **High throughput in-vitro early toxicity and off-target liability assays to rapidly identify limitations of novel thyromimetics**
Sheraz GUL – (MC substitute Germany) Fraunhofer Institute, Hamburg (Germany)

12.45 OC8 **Cytotoxic Effect of Zn(II)/Au(I), Zn(II)/Ag(I) And Ru(III) Complexes with Schiff Bases in Human Osteosarcoma Cellstitle**
Radostina ALEXANDROVA – (MC member Bulgaria) Bulgarian Academy of Sciences (Bulgaria)

13.00 OC9 **Multitarget Covalent Inhibitory Property of Klavuzons**
Ali ÇAĞIR – Izmir Institute of Technology (IYTE), İzmir (Turkey)

13.15 *Lunch and poster session*

Session II "Multi-targeting agents against neurodegenerations"
Moderator: Fernanda BORGES – (Vice-Chair and MC member Portugal) University of Porto (Portugal)

14.45 OC10 **Expanding the medicinal chemistry Multi-Target Directed Ligands toolbox for neurodegeneration: rationally designed fragment, hybrids and conjugate small molecules**
Maria Laura BOLOGNESI – (STSM coordinator) University of Bologna (Italy)

15.00 OC11 **Natural Product Hybrids are Potent Neuroprotectants in Vitro And in Vivo: Multi-Target Ligands with Unknown Targets?**
Michael DECKER – University of Würzburg (Germany)

15.15 OC12 **Targeting Alzheimer's Disease with 4-Aminochromane Derivatives**
Anthony BURKE – University Evora (Portugal)

15:30 OC13 **Combination of Adenosine Antagonism with Additional H3R Antagonism for the Treatment of Parkinson's Disease**
Holger STARK – (MC member Germany) Heinrich Heine University of Duesseldorf (Germany)

15.45 OC14 **Histamine H3 receptor antagonism as a valuable component for multitarget directed ligands (MTDL)**
Wieslawa Agnieszka FOGEL – (MC member Poland) Medical University of Lodz (Poland)

16.00 OC15 **Repurposing Compounds Designed to Treat Mental Diseases as Potential Drugs Against Neurodegenerative Diseases**
Agnieszka A. KACZOR – Medical University of Lublin (Poland)

16.15 OC16 **Mapping the chromone-3- phenylcarboxamides pharmacophore: structure-activity-toxicity and efflux transport studies**
Francesco MESITI – Università "Magna Græcia" di Catanzaro (Italy)

16:30 *Coffee break*

Session III "Multi-targeting agents against vascular diseases or oxidative stress"
Moderator: Florence McCARTHY – (MC member Ireland) University College, Cork (Ireland)

16.45 OC17 **Interplay between superoxide dismutase, catalase and peroxidase activities for salen-manganese complexes**
Marcelino MANEIRO – University of Santiago de Compostela, Santiago de Compostela (Spain)

17.00 OC18 **Mitochondriotropic Cinnamic Acid Antioxidant Improves Cellular Resistance to Stress**
Ricardo AMORIM – University of Porto, Porto (Portugal)

17.15 OC19 **Vascular effects of pioglitazone related to perivascular adipose tissue (PVAT)**
Deniz KALELİ DURMAN – Istanbul University, İstanbul (Turkey)

17.30 OC20 **The journey from ethnobotanical studies to pharmacological target: The effect of imperatorin and isoimperatorin on penile relaxation**
Nazlıcan BELEN – Ege University, Izmir (Turkey)

17.45 OC21 **A Potential Repositioning of Trimetazidine for the Treatment of Bladder Dysfunction: Effects on Mouse Detrusor Contractility and Cytoprotection**
Yeşim KAYA YAŞAR – Karadeniz Technical University, Trabzon (Turkey)

18.15 *Transfer to gala dinner (optional)*

Friday, March 6th 2020

Session IV: "New methods in Drug Discovery, in silico pharmacology and drug repurposing"
Moderator: Holger STARK – (MC member Germany) Heinrich Heine University of Duesseldorf (Germany)

9.00 OC22 **A Lab-on-a-Molecule with an Enhanced Fluorescent Readout on Detection of Three Chemical Species**
David C. MAGRI – (MC member Malta) University of Malta (Malta)

9.15 OC23 **New screening libraries arising from the Prestwick Chemical Drug Library**
Marie Louise JUNG – Prestwick Chemical, Strasbourg (France)

9.30 OC24 **Lead to Target: A Computational Approach to Identify Possible Protein Targets of Molecules with Known Experimental Biological Activity**
Sérgio F. SOUSA – University of Porto (Portugal)

9.45 OC25 **Drug design in silico: dream, (next) future or even coming reality?**
Diego LIBERATI – National Research Council of Italy @ Politecnico di Milano (Italy)

10.00 OC26 **Computational Approaches in Multi-target Drug Discovery**
Rita C. GUEDES – (MC member Portugal) University of Lisbon (Portugal)

10.15 OC27 **Multi-Target Ligand Design is Ripe for New Methods**
Alfonso T. GARCÍA-SOSA – (MC member EE, communication manager) University of Tartu (Estonia)

10.30 OC28 **New Inhibitors of the BmrA Pump Identified Through Virtual Screening**
Onur SERÇİNOĞLU – Recep Tayyip Erdogan University, Rize (Turkey)

10.45 *Coffee break*

11.00 **Round table on perspectives of the MuTaLig community**
Moderator: Stefano Alcaro (CA15135 Chair) - Università "Magna Græcia" di Catanzaro (Italy)
Antonio LUPIA – (FAD manager) Net4Science academic spinoff, Catanzaro (Italy)
Fernanda BORGES – (Vice-Chair and MC member Portugal) University of Porto (Portugal)
Danijel KIKELJ – (WG1 leader) University of Ljubljana (Slovenia)
Alfonso T. GARCÍA-SOSA – (MC member EE, communication manager) University of Tartu (Estonia)
Maria Laura BOLOGNESI – (STSM coordinator) University of Bologna (Italy)

12.15 **Concluding remarks and best poster awarding ceremony**
Günay YETİK-ANACAK (MC member Turkey and local organizer) – Ege University (Turkey)

12.45 Transfer and visiting *Yesilova Höyük Neolithic Settlement and Farewell cocktail*

15.15 Transfer for daily tour (optional)

Oral Communications

Short communication 1 (WG1)

Toward Multitarget DNA Gyrase Inhibitors Possessing Antibacterial Activity

T. Tomašič,[1] J. Ilaš, N. Zidar, L. Peterlin Mašič, A. Zega, M. Durcik, Ž. Skok and D. Kikelj

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Bacterial DNA gyrase is an ATP-fueled heterotetrameric protein, composed of two A subunits (GyrA) and two B subunits (GyrB) that is essential for cell viability because it introduces negative supercoils in DNA in front of the replication fork. It is structurally similar to topoisomerase IV (containing Par C and ParE subunits) that is responsible for DNA decatenation. The GyrA /ParC subunits are the target of fluoroquinolone antibiotics, while the GyrB/ParE are a target of novobiocin and a number of recently reported gyrase inibitors. Noviobicin, discovered in the mid-1950s, was withdrawn from the market primarily due to its toxicity and due to the high resistance development and so far, no GyrB inhibitor has been introduced into the clinic. Generally, it is assumed that dual targeting could reduce bacterial resistance because mutations at two different sites are less probable to occur than single mutation in GyrA and GyrB sites.

We will present our efforts and progress made during the last few years toward dual DNA gyrase / topoisomerase IV inhibitors and dual gyrase A / gyrase B inhibitors which display in vitro antibacterial activity.

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[1] Gjorgjieva, M.; Katsamakas, S.; Barančokova, M.; Tomašič, T.; Ilaš, J.; Peterlin Mašič L.; Kikelj, D. *J. Med. Chem.* **2016**, *59*, 8941−8954

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Short communication 2 (WG1)

Repositioning of [1,6] naphthyridines as antiviral agents on HSV-1

Virginia Spanò,[a] Ilaria Frasson,[b] Marilia Barreca,[a] Anna Carbone,[a] Maria Valeria Raimondi,[a] Stefano Alcaro,[c,d] Sara N. Richter,[b] Paola Barraja,[a] Alessandra Montalbano,[b]

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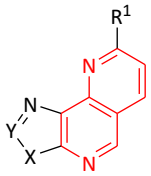
Herpes simplex virus 1 (HSV-1) is a large, double-stranded DNA virus that infects epithelial cells and establishes a latent infection in sensory ganglia of the host, from where it periodically reactivates causing recurrent lesions at the site of primary infection. More-severe diseases caused by HSV-1 infection include encephalitis, meningitis, and blinding keratitis. Current clinical therapies rely on nucleoside drugs to ameliorate primary infections and to inhibit or reduce the symptoms of reactivations. Since these treatments do not fully suppress viral shedding, long term therapy is necessary, leading to the development of drug-resistant strains[1]. Therefore, it has become mandatory to explore non-nucleoside cores to develop innovative antiherpetic agents. In the last years, our efforts aimed at the study of heterocyclic scaffod as precursor of new potential photosensitizers[2]. We have recently synthesized [1,2,3]triazolo (**1**), [1,3]oxazolo (**2**)[3] and [1,3]thiazolo[1,6]naphthyridines (**3**) endowed with promising singlet oxygen sensitizer properties, without cytotoxic effect on human cell lines in the absence of UV irradiation.



1 X=N-R; Y=N **2** X=O; Y=C-R **3** X=S; Y=C-R

Since [1,6]naphthyridines have been recently proposed as a novel class of anti-HSV agents[3], we tested our tricyclic systems on HSV-1 lytic cycle, thus undertaking a repositioning study of those compounds as antiviral agents. Selected derivatives displayed a remarkable inhibition of the viral cycle, with IC_{50} values in the low nanomolar range. The viral step hindered by the most active compounds is now under investigation. Moreover, the antiviral effect of tested compounds will be checked also on HSV-1 strains, wild type and drug resistant, isolated from patients by the Microbiology and Virology Unit of Padova Teaching Hospital.

References

[1] Bernier, K.M.; Morrison, L.A. *Antiviral Res*. **2018**, *156*, 102-106. [2] see for example: Spanò, V.; Giallombardo, D. ; Cilibrasi, V.; Parrino, B.; Carbone, A.; Montalbano, A.; Frasson, I.; Salvador, A.; Richter, S. N.; Doria, F.; Freccero, M.; Cascioferro, S.; Diana, P.; Cirrincione, G.; Barraja, P. *Eur. J. Med. Chem.* **2017**, *128*, 300–318; [3]Frasson, I.; Spanò, V.; Di Martino, S.; Nadai, M.; Doria, F.; Parrino, B.; Carbone, A.; Cascioferro, S. M.; Diana, P.; Cirrincione, G.; Freccero, M.; Barraja, P.; Richter, S. N.; Montalbano, A. *Eur. J. Med. Chem.* **2019**, *162*, 176–193.[3] Bernardino, A. M. R.; Azevedo, A. R.; Pinheiro, L. C. S.; Borges, J. C.; Paixao, I. C. P.; Mesquita, M.; Souza, T. M. L.; dos Santos, M. S. *Org Med Chem Lett* **2012**, *2*, 1–7.

Short communication 3 (WG1)

Indolocarbazole maleimides with dual inhibition of kinases and topoisomerase I

Louise N.Cooney,[a] Kevin O'Shea,[a] Hannah Winfield,[a] Michael Cahill,[a] Larry Pierce,[a] Florence O. McCarthy[a]

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Since the discovery of the natural product indolocarbazole staurosporine (**1**), many analogues have been synthesised to obtain higher potency and selectivity. Modification of the sugar and headgroup components has led to compounds for which different modes of biological action are reported. One such example rebeccamycin (**2**) is a highly effective -+inhibitor of topoisomerase I, and other examples have reported polypharmacology including the inhibition of multiple protein kinases and other clinical targets in cancer therapy. The recent FDA approval and progression to market of Rydapt (midostaurin **1**, R = Benzoyl) identifies the indolocarbazoles as a rich source of pharmaceutical candidates.

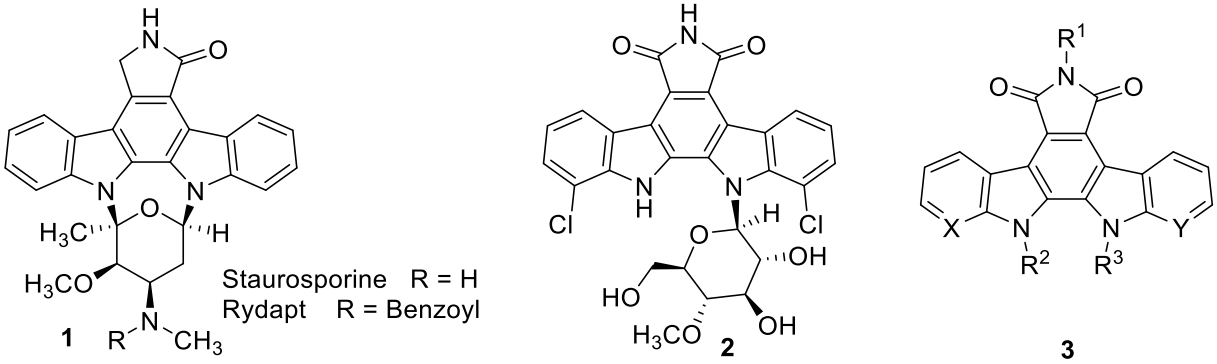

Figure 1: Structures of staurosporine, rebeccamycin and a representative indolocarbazole

Our work is focused on discovery of highly potent anticancer agents and to date we have produced several novel heterocycles as a replacement for the lactam/maleimide in structures (**1**/**2**).[1,2,3] We hereby describe indolocarbazoles which possess dual topo/kinase inhibition through modification of the framework utilising substituents on the indole and maleimide rings (**3**). Anticancer characterisation via the NCI 60 cell line screen has identified nanomolar inhibition of cancer cell growth and screening of kinase and topoisomerase inhibition has identified potential clinical targets.

References

[1] Pierce, L. T.; Cahill M. M.; McCarthy, F. O. (a) Synthesis of novel 3,4-diaryl-5-aminopyrazoles as potential kinase inhibitors. *Tetrahedron,* **2011**, *67 (25),* 4601-4611. (b) Design and synthesis of novel 5,6-bisindolylpyrimidin-4-ones structurally related to ruboxistaurin (LY333531). *Tetrahedron,* **2010**, *66 (51)*, 9754-9761.

[2] Pierce, L. T.; Cahill M. M.; Winfield, H. J.; McCarthy, F. O. Synthesis and identification of novel indolo[2,3-*a*]pyrimido [5,4-*c*]carbazoles as a new class of anti-cancer agents. *European Journal of Medicinal Chemistry*, **2012**, *56*, 292-300.

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Short communication 4 (WG3)

Epigenetic multitargeting in cancer: design, synthesis and biological evaluation of novel inhibitors

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Epigenetic pathways are being recognized as determinants to cancer development and progression. The overexpression of EZH2, the catalytic subunit of PRC2, that catalyzes the trimethylation of lysine 27 in Histone 3 (H3K27me3), is implicated in the development and progression of a variety of cancers with the worst prognosis.[1] Histone deacetylases (HDACs) remove acetyl groups from histones that regulate the expression of different proteins involved in cancer.[2] Evidences of synergistic effects of HDAC and EZH2 inhibition have been reported for cancer treatment.[3] Thus, the development of selective small-molecule inhibitors is currently a promising research challenge for drug discovery.

A combination of state-of-the-art techniques, from computational drug design to synthetic methodologies and biological testing, is being used to develop the new molecules. We performed a computer-aided drug design campaign to design new EZH2-HDAC inhibitors using LigandScout.[3] A panel of unique pharmacophore models was generated, validated and optimized. The prioritized models were used for two hit finding campaigns: Virtual Screening and *De Novo* Design. For the VS approach, several databases (e.g., DrugBank, NCI, MuTaLig Chemotheca, and in-house libraries) were computed and screened. Interesting virtual hit molecules with high inhibition potential were found and tested in order to determine their EZH2 and HDAC profiles. Notably, we found several hits with inhibition rates comparable to the reference compounds. In parallel, we started a *De Novo* Design campaign based on selected pharmacophore models and we found a new scaffold for EZH2 inhibitors. Those from *de novo* design are being synthesized. The potential toxicity issues are also being assessed through ADMET studies. Finally, selectivity and binding mode of the most promising compounds will be elucidated. The best drug candidates are expected to proceed to *in vivo* testing.

Acknowledgements: This work was supported by grant PD/BD/128320/2017 from Fundação para a Ciência e a Tecnologia (FCT) and projects UID/QUI/00100/2019, UID/DTP/04138/2019, PTDC/QUI-QAN/32242/2017, and SAICTPAC/0019/2015, funded by national funds through FCT and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This communication is based upon work from COST Action CA15135, supported by COST.

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Short communication 5 (WG2)

The effect of New Synthesized Resveratrol Derivate H1 on angiogenesis

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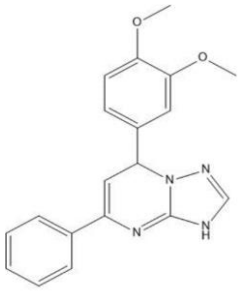
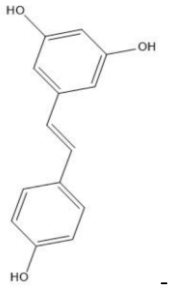
Angiogenesis is crucial in tumor invasion and metastases thus inhibition of angiogenesis is important in cancer treatment (1). Resveratrol, a polyphenolic compound naturally found in peanuts, grapes, red wine, and some berries, can inhibit angiogenesis in higher concentrations (2). However bioavailability of resveratrol is low and thats why several studies aimed to synthesis new resveratrol derivatives (3). In this context, new triazolopirimidin derivate resveratrol analog (H1) was synthesized by our group. We have tested the effects of H1 on angiogenesis by in vitro and in vivo assays. MTT assay were performed in BAEC ($6x10^4$/ml cells in DMEM supplemented with 10% FBS) after three days of incubation with H1 or resveratrol (0.78 - 200 µM) . IC50 values calculated from dose-response curve for cell survival. Both H1 and resveratrol inhibited endothelial cell proliferation. However inhibitory effect of H1 was more potent than resveratrol (H1 IC50=20.315 µM, Resveratrol IC50=34,317 µM).

The effect of resveratrol or H1 (10 µM) on tube formation was tested on BAECs ($5x10^4$/well) plated in growth factor-reduced Matrigel. The degree of tube formation was quantified by measuring the length of tubes from each well using the Image J program. Both Resveratrol and H1 inhibited formation of capillary-like tubes dose dependently. Partly inhibition at 10 µM and complete inhibition at 100 µM was observed for both molecules. However the inhibition of tube formation at 10 µM was tended to be stronger for H1 than Resveratrol. The effect of RVT or H1 (25 or 50 µmol/L) on migration was documented by photos taken 7 or 24 h after scraping of BAEC. The percentage of wound closure was calculated by Image J program. Both H1 and Resveratrol treatments (25 or 50 µM) inhibited cell migration compared with control significantly ($p<0.001$).

Our result showed a strong antiangiogenic effect of the new resveratrol derivatives. These derivatives may provide potential benefits for excessive angiogenesis associated diseases specially cancer.



H1



Resveratrol

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Short communication 6 (WG2)

A comparative study of the antiangiogenic activity of hydroxytyrosyl alkyl ethers as multitargeted bioactive compounds

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The phenolic compound hydroxytyrosol is present in its free form or as derivatives in extra virgin olive oil. Hydroxytyrosol is considered one of the bioactive compounds responsible of the health benefits of olive oil intake. The anti-angiogenic effects of hydroxytyrosol *in vitro*, *ex vivo* and *in vivo* have been previously shown. Among several tested derivatives, hydroxytyrosyl ethyl ether has been shown to have more potent anti-angiogenic effects than hydroxytyrosol. The present study is based on the following working hypothesis: the length of the aliphatic chain in hydroxytyrosyl alkyl ethers could be determinant in their overall potential anti-angiogenic activities. This was the case for their effects on endothelial cell growth. Hydroxytyrosyl hexyl ether was the best of the tested compounds based on its effects on an array of *in vitro* angiogenesis assays, as well as on its effects on the *in vivo* chorioallantoic membrane assay.

Short communication 7 (WG2)

High throughput *in-vitro* early toxicity and off-target liability assays to rapidly identify limitations of novel thyromimetics

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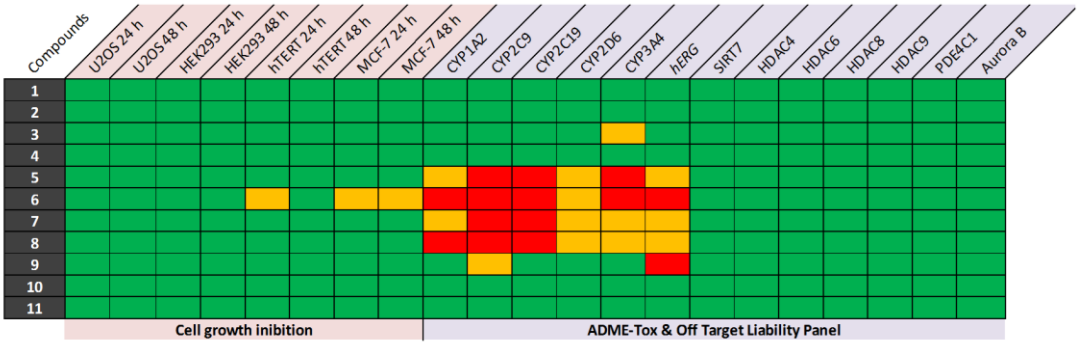
In order to rapidly identify the phenotypic profile and possible off-target liability effects of novel synthesized thyromimetics, we performed *in-vitro* screening on a new small library of synthetic thyromimetics. We screened compounds in a comprehensive panel of early toxicity assays comprising cytotoxicity against 4 different cell lines (osteosarcoma, U2OS; lung fibroblast, hTERT; human breast adenocarcinoma, MCF7; human embryonic kidney, HEK293), *h*ERG liability, cytochrome P450 inhibition (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isoforms), and off-target liability against selected proteins (Aurora B kinase and phosphodiesterase PDE4C1) and epigenetic enzymes (HDAC4, HDAC6, HDAC8, HDAC9 & SIRT7). All the compounds were screened at 10 µM in at least triplicate using these *in-vitro* assays. The data generated will help investigators to accelerate the development of pharmacological strategies to identify lead compounds with increased chances of progression in the drug discovery value chain.



% Inhibition at 10 µM [compound]	Classification
<50	Acceptable profile
51-90	A flag that requires remedial action
>91	Major issue that requires significant attention

Figure 1: Off-target and ADME-Tox profile of selected compounds with traffic-light characterization.

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Short communication 8 (WG2)

Cytotoxic effect of Zn(II)/Au(I), Zn(II)/Ag(I) and Ru(III) complexes with schiff bases in human osteosarcoma cellstitle

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The treatment of osteosarcoma continues to be among the major challenges for modern oncology, medical chemistry and oncopharmacology.

The aim of our study was to evaluate the cytotoxic effect in Saos-2 human osteosarcoma cells of the following two groups of compounds: three heterometallic complexes of {Zn(II)/Au(I)} and {Zn(II)/Ag(I)} with Schiff bases resulted from the condensation reaction between salicylaldehyde with ethylenediamine (H_2Salen), N,N-dimethyl-ethylenediamine (HSaldmen), and respectively, 2-aminomethyl-pyridine (HSalampy) (Group A); three Ru(III) complexes with Schiff bases obtained by the condensation reaction between o-vanillin and ethylenediamine (H2Valen), 1,3-diaminopropane (H_2Valpn), and 1,2-phenylenediamine (H_2Valphen) (Group B).

The investigations were carried out by short-term experiments (3h – 72h, with monolayer cell cultures) and long term experiments (> 20 days, with 3D colonies of cancer cells) using methods with different molecular/cellular targets and mechanisms of action such as MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, hematoxylin and eosin staining, double staining with acridine orange and propidium iodide, AnnexinV/ICH - DAB chromogene method and 3D cell colony-forming technique. The compounds were used at a concentration range of 0.05 - 10 µg/ml (for Group A) and 0.1-100 µg/ml (for Group B).

The results obtained revealed that all complexes examined decreased viability of the treated cells in a time- and concentration-dependent manner as well as their ability to form 3D colonies in a semisolid medium. {Zn(II)/Au(I)} and {Zn(II)/Ag(I)} complexes with Schiff bases were found to be more pronounces cytotoxic agents as compared to Ru(III) complexes. The free ligand H2Salen did not show cytotoxic activity.

Acknowledgements: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by DCM # 577 / 17.08.2018; National Science Fund in Bulgaria – Grant №ДКОСТ 01/16 from 17.08.2017 and Grant №ДКОСТ 01/10 from 22.10.2018; bilateral project between Bulgarian Academy of Sciences and Romanian Academy; COST Action CA15135 "MuTaLig" and COST Action CA16119 "CellFit".

Short communication 9 (WG1)

Multitarget Covalent Inhibitory Property of Klavuzons

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Covalent inhibitors hold more than 30% share of medicines sold in the market. Their covalent bond forming capability with intercellular targets make them stronger inhibitor compared to noncovalent inhibitors. However there are serious concern about the selectivity of the covalent inhibition. Most of the time screening of the potential inhibitory effects of these molecules toward the intracellular targets are disregarded.

Klavuzons are derivatives of 6-(naphthalene-1-yl) substituted 5,6-dihydro-*2H*-pyran-2-one pharmacophore first time reported by our group in 2008. It is believed that Michael acceptor property of 5,6-dihydro-*2H*-pyran-2-one is responsible for the biological activity. So far, 29 derivatives of klavuzons have been synthesized and their inhibitory properties were evaluated toward 29 different intracellular targets including Topo I, HDAC1, SIRT1, CRM1, KRasG12C and 24 different kinases. [1-3] In terms of target selectivity, it has been found that Topo I, SIRT1, CRM1 and KRasG12C are valuable targets for klavuzons derivatives. Especially CRM1 is a special target due to the its inhibitory effect can be seen at low nanomolar concentrations. On the other hand, we believe that changing the size and positions of the substituents present over naphthalene-1-yl group may tune the selectivity to a target macromolecule. In silico studies revealed that both enantiomers can possess some degree of activity depending on the type of the substituents presents over naphthalene-1-yl group. Antitproliferative effect of the klavuzons over cancer cells have been shown in 2D and 3D cell cultures. Antitumor activity of one klavuzon derivative in patient derived xenograft model has also been shown.

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Short communication 10 (WG1)

Expanding the medicinal chemistry Multi-Target Directed Ligands toolbox for neurodegeneration: rationally designed fragment, hybrids and conjugate small molecules

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Neurodegenerative diseases are some of the leading medical and societal challenges faced by the global population. Although their basic molecular mechanisms are not fully understood, it is widely recognized that their pathogenesis involves an intricate array of concomitant, intertwined processes. Thus, it may be unlikely that drugs acting on single targets can effectively control them. In 2008, we proposed multitarget-directed ligands (MTDLs) for their potential to effectively cure such disorders.[1] In the years we have applied these concepts to the development of small molecules that differ for their intrinsic features and design concepts: i.e. fragments, hybrids and conjugates.[2] Here, we discuss pro and cons with examples of each approach and a provide a perspective on the implications for further clinical translation.

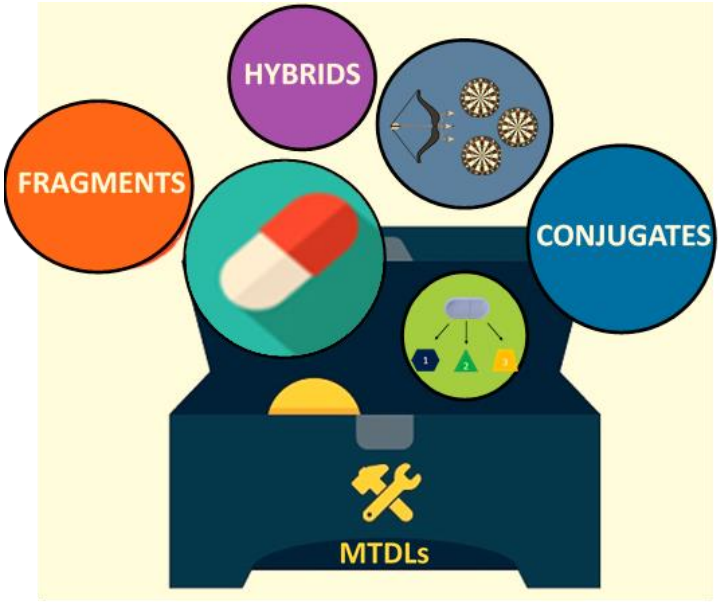

Figure 1: Multi-Target Directed Ligands toolbox for neurodegeneration

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Short communication 11 (WG1)

Natural product hybrids are potent neuroprotectants in vitro and in vivo: multi-target ligands with unknown targets?

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Flavonoids and flavonolignans have for a long time been described as "antioxidants" with putative neuroprotectivity. Nevertheless, their lack of "druggability" and seemingly unspecific modes of action has not made them attractive starting points for drug discovery in modern medicinal chemistry. But in fact, chemical modifications, such as combination with phenolic acids, can lead to significant improvement of in vitro properties with multi-modal activities, some of them highly sensitive to chemical modifications.[1] We regioselectively synthesized the 7-*O*-esters of taxifolin and silibinin, respectively, and phenolic acids showing pronounced overadditive neuroprotective effects against oxytosis, ferroptosis and ATP depletion in murine hippocampal neuron HT22 cells, and reduced LPS-induced neuroinflammation in BV-2 microglia cells.[2] In all *in vitro* assays, the 7-*O*-esters showed strong overadditive activity.[1,2]

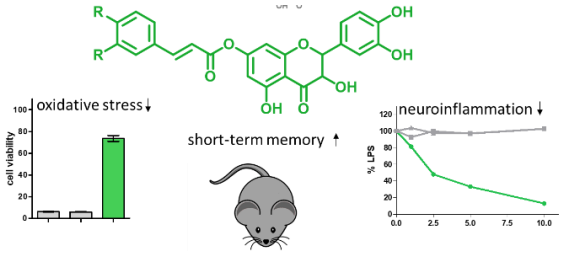

Figure 1: Structure of flavonoid hybrids and resulting over-additive biological effects.

In vivo studies confirm these overadditive effects: Treatment in an Alzheimer`s disease mouse model (based on the injection of oligomerized $A\beta_{25-35}$ peptide into the brain to cause neurotoxicity and subsequently memory deficits) with 7-*O*-cinnamoyltaxifolin or 7-*O*-feruloyltaxifolin resulted in improved performance in an assay for short-term memory.[2]

These results highlight the benefits of natural product hybrids for multi-target drug discovery due to their pharmacological profile that is distinct from the individual natural components.

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Short communication 12 (WG1)

Targeting Alzheimer's Disease with 4-Aminochromane Derivatives: Single and Dual Inhibitors

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In the quest to discover a cure for Alzheimer's disease, the *Multi-Target-Directed Ligand* approach has been found to be a successful paradigm. Both compounds **1** and **2** were found to be good examples of this approach [1].

Heterocyclic units are common in many commercial drugs, within this category those containing a chromane unit are particularly interesting, and we have demonstrated that compounds **4** show appreciable BuChE inhibitory effects (but weak MAO activity), with the amino analogues **5** under investigation [2]. With a view to harnessing the 4-aminochromane unit as a key component for the creation of novel dual inhibitor ligands, we are currently developing the novel hybrid class **3** (Fig. 1). In this presentation we will discuss the synthesis and bioassays of compounds **3** and **4** as well as the insights obtained from molecular docking and STD-NMR.

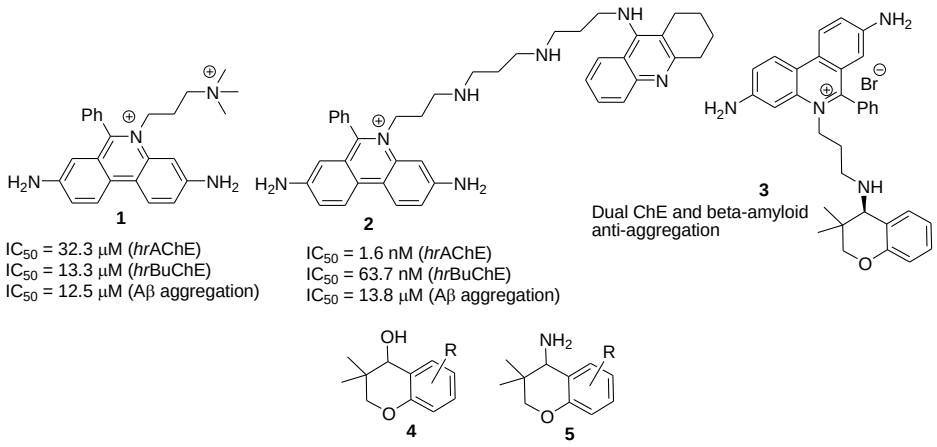

Figure 1: Single and Dual-inhibitors – Known and under investigation.

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Short communication 13 (WG2)

Combination of adenosine antagonism with additional h_3r antagonism for the treatment of parkinson's disease

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Adenosine A_1/A_{2A} receptors ($A_1R/A_{2A}R$) modulating dopamine neurotransmission represent targets in non-dopaminergic treatment of motor disorders like Parkinson's disease (PD). Multi-targeting ligands (MTLs) were developed to achieve more comprehensive PD therapies addressing also comorbid symptoms, such as sleep disruption. Recognizing the wake-promoting capacity of histamine H_3 receptor (H_3R) antagonists in combination with the "caffeine-like effect" of A_1R/ $A_{2A}R$ antagonists, a series of $A_1R/A_{2A}R/H_3R$ MTLs was designed, where a H_3R pharmacophore was introduced into an adenosine antagonist core structure. These MTLs showed distinct receptor binding profiles with overall nanomolar H_3R affinity ($K_i < 55$ nM). Improvement of L-DOPA-induced dyskinesia in rats was observed after administration of compound **4** (1 mg kg^{-1}, i.p.). Compound **12** (2 mg kg^{-1}, p.o.) was able to increase wakefulness in mice. Designed $A_1R/A_{2A}R/H_3R$ MTLs, described for the first time and showing distinct affinity profiles, may represent innovative pharmacological tools and a novel approach for PD therapy.

Acknowledgement: This work was kindly supported by COST Actions CM1103, CA15135, CA18133 as well as DFG INST 208/664e1 FUGG (Germany) and Inserm U 628.

Short communication 14 (WG2)

Histamine H3 receptor antagonism as a valuable component for multitarget directed ligands (MTDL)

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Histamine H3 receptors (H3R), located on histaminergic and other neurotransmitter neurons, serve to control as auto- or heteroreceptors, respectively, the release of histamine, as well as acetylcholine, dopamine, noradrenaline, serotonin, glutamate and substance P. An inhibition of these receptors increases the intrasynaptic prevalence of neurotransmitters, what should be perceived as valuable, regarding their deficits in numerous neurodegenerative diseases. Therefore, in many multitarget directed ligands (MTDL), that have been designed and synthesized to alleviate the symptoms of the diseases, H3R antagonism/inverse agonism has been combined with either inhibitory moieties against f.ex., monoamine oxidase, monoamine transporters, histamine *N*-methyltransferase, beta-amyloid aggregation or others, e.g agonism to dopamine receptors((cf. Khanfar et al., 2016).

Our study project focused on ST-2309, an MTDL candidate, which is characterized by a high antagonist potency at the recombinant human H3 receptor (Ki: 12.5 ±0.33 nM), which was subchronically administered to Wistar rats to investigate its effects on crossing the blood-brain-barrier. It was assumed that if the compound, administered to rats, would cross the blood-brain-barrier and block the cerebral H3 receptors, histamine would be released and suppress food intake in rats by activating H1 receptors (Pan et al.,2006). That in vivo test was performed in metabolic cages where rats were individually kept, enabling feed and water consumption measurements. ST-2309, 3 mg/kg body wt was subcutaneously administered for 4 consecutive days and ciproxifan, 3mg/kg kg body wt s.c. was used as a reference agent. The treatment was preceded by a 3-day control drug-free, period, with feed and water consumption recorded at 9 am each morning. In the following days injections administered always after consumption records. On the fifth day, after the last record, the rats received one more drug injection and were sacrificed 1 hr later, with their brains being collected for analysis.

An analysis of results demonstrated that both groups of the H3R blocker-treated rats had reduced their feed/water consumption – proving the drugs to have entered their brains. Moreover, a postmortem analysis of the enzyme activities related to histamine degradation, i.e. histamine *N*-methyltransferase and monoamine oxidase B, disclosed a significant 20 % inhibition of HMT by ST-2309. Studies are underway to disclose the cerebral concentrations of neurotransmitters and metabolites upon the treatment. In summary, the ST-2309 compound seems to be a fairly promising MTDL combining both, 3HR and HMT inhibition.

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Short communication 15 (WG1)

Repurposing compounds designed to treat mental diseases as potential drugs against neurodegenerative diseases

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Central nervous system (CNS) diseases, being the cause of 2.1% of all deaths, have a relatively high prevalence. Furthermore, it has been estimated that CNS disorders belong to the most costly medical conditions and are among the leading public health problems in the European Union and worldwide.

We used rational structure-based design methods to develop multi-target ligands of aminergic G protein-coupled receptors which can be used to treat mental diseases, such as schizophrenia, depression or anxiety. Detailed investigation of *in vitro* and *in vivo* profiles of the selected compounds indicated that they may be repurposed for the treatment of neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease.

It was determined that the selected compounds displayed an increase in proliferation of mouse hippocampal neuron cells. In the case of neuroblastoma cells, there is no increase in cell proliferation. Moreover, the cells incubated with the compounds are more elongated, characterized by longer dendrites as compared to the control. Next, the compounds lower the levels of reactive oxygen and nitrogen species (ROS and NOS, respectively) and in the test using the Ca^{2+} probe cause the decrease of calcium level with the increasing cell incubation time. The compounds do not cause the influx of calcium ions into the cell, which also protects the cell against the excitotoxicity process.

In the behavioral tests the selected compounds exhibit pro-cognitive properties in the passive avoidance test and novel object recognition test in mice both after acute and chronic administration.

Finally, the PASS software was applied to search for possible additional molecular targets to explain the observed *in vitro* activity of the compounds and the identified targets are under experimental validation.

Short communication 16 (WG1)

Mapping the chromone-3-carboxamide pharmacophore: structure-activity-toxicity and efflux transport studies

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Parkinson disease is a neurodegenerative disorder principally characterized by the neuronal loss in the *substancia nigra*, which causes striatal dopamine deficiency responsible for several of motor symptoms, including bradykinesia, tremor and postural instability[1]. Among all the PD drugs used in therapy to restore the dopamine levels, in the last few decades the MAO-B inhibitors (iMAO-B) have received a lot of attention, since they showed neuroprotective properties[2]. Then, considering the urgent need of new therapeutic solutions for PD, the side effects of the drugs in therapy and the capability of iMAO-B to act as disease modifying agents[3], our project is focused in the design and development of new iMAO-B. Previously, our group validate chromone- and coumarin- 3-carboxamides as relevant iMAO-B lead compounds[3, 4].However, to improve their drug-like properties it was found relevant to map the pharmacophore through a diversity of structural modifications on the benzopyrone ring. The project started on the selective chromone inhibitors Cr-1 (*h*MAO-B IC_{50} 0.67nM) and Cr-2 (*h*MAO-B IC_{50} 0.40 nM)[4] and to assess the role of the benzopyran heteroatom, the double carbon bond, and the carbonyl group, several types of compounds were synthetized and biologically evaluated. In particular, for quinolone derivatives the tautomerism relevance on MAO-B inhibition was figured out. Finally, cytotoxicity and efflux transport studies were performed for the most promising compounds.

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Short communication 17 (WG1)

Interplay between superoxide dismutase, catalase and peroxidase activities for Salen-Manganese complexes

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Reactive oxygen species (ROS) are generated as by-products of mitochondrial electron transport of aerobic respiration. An imbalance between antioxidants and oxidants could determine the extent of cell damage. In this context, efforts have been directed toward the search of antioxidant enzyme mimics. Salen-manganese complexes emerge as effective multi-target ROS scavengers that can behave as superoxide dismutase, catalases or peroxidases mimics. However, the mechanism of action of this type of systems remains exiguously understood.

During studies aimed to getting a better understanding of the processes involved in the antioxidant behavior of these biomimetic models, we found that the reaction mechanism versus a common oxidant as hydrogen peroxide is strongly dependent on the conditions. Solvent media, illumination conditions and other factors may vary not only the antioxidant activity but also the reaction mechanism itself.[1,2] The whole set of kinetic experiments indicate an interplay between SOD, catalase and peroxidase activities as the active catalase mimic is formed after radical scavenger reaction (Fig. 1).

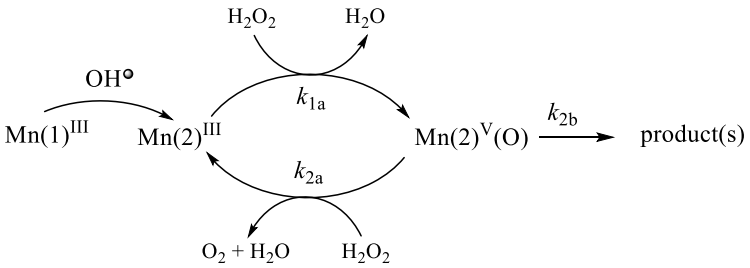

Figure 1: Proposed mechanism for radical scavenger and catalytic decomposition of H_2O_2 by the biomimetic models.

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Short communication 18 (WG2)

Mitochondriotropic Cinnamic Acid Antioxidant Improves Cellular Resistance to Stress

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Background: Mitochondria are key organelles involved in cellular survival, differentiation and cell death induction. In this sense, alterations in mitochondrial morphology and/or function are involved in stress-induced adaptive pathways, priming mitochondria for mitophagy, or apoptosis induction. The concept of hormesis states that exposure of cells to a low-dose of a potentially harmful stressor (*e.g.* ROS) triggers an adaptive response that renders them less sensitive to subsequent exposures. Within this context, mitochondrial hormesis ("mitohormesis") occurs when low-intensity stress triggers a retrograde cascade that induces protective (non)-mitochondrial adaptations to restore and/or maintain cellular homeostasis. Compatible with a potential hormesis mechanism, phytochemicals, which are antioxidant and/or redox-active constituents of the normal human diet, are able to induce adaptive cellular stress response pathways. By conjugation with tetraphenylphosphonium cation (TPP^+), we recently developed mitochondria-targeted antioxidants based on caffeic acid ($AntiOxCIN_4$). Our current hypothesis is that $AntiOxCIN_4$ can increase the cellular resistance to stress by modulating signaling and metabolic pathways through a process of "mitohormesis".

Material and Methods: Herein, we studied the effects of the novel mitochondriotropic agent ($AntiOxCIN_4$) on human hepatoma-derived HepG2 cell line by measuring its effects on mitochondrial physiological parameters, namely on oxidative stress markers, mtDNA copy number, oxygen consumption, antoxidant defense system and mitophagy after 48h of treatment. Data presented are means ± SEM of four independent cell experiments. Significance was accepted with $P<0.05$.

Results: After an initial decrease in O_2 consumption paralleled by a moderate increase in ROS levels, $AntiOxCIN_4$ increased 1.6-fold basal respiration and 1.2-fold the extracellular acidification. A modulation of the endogenous antioxidant defense system and mitochondrial biogenesis was observed by a significant increase of 20% in GSH content and in the mtDNA copy number. $AntiOxCIN_4$ treatment promoted an efficient removal of potential damaged mitochondria by triggering cellular quality control mechanisms, such as autopaghy and/or mitophagy, while mantained its remarkable antioxidant properties

Conclusions Mitochondriotropic antioxidants based on dietary scaffolds, such as phenolic acids, can modulate cellular metabolic activity throughout the improvement of mitochondrial function, which increase their usefulness as therapeutic agents in the treatment of oxidative stress-related conditions.

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Short communication 19 (WG2)

Vascular effects of pioglitazone related to perivascular adipose tissue (PVAT)

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Perivascular adipose tissue (PVAT) is a local adipose tissue layer that surrounds the vessels. It produces various adipokines with paracrine effects on the adjacent layers of the vasculature and thus, influencing vascular function. It's observed that pathophysiological conditions such as obesity, insulin resistance and type-2 diabetes (T2D) may cause PVAT dysfunction that leads to vascular disease. [1-2] Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors which are members of hormone receptor superfamily and are divided into three subtypes; PPARα, PPARβ/δ and PPARγ. PPARγ is highly expressed in adipose tissue and has a role in insulin sensitivity, thus it's an interesting therapeutic target. Pioglitazone is a thiazolidinedione (TZDs), a selective PPARγ agonist used in the treatment of T2D. Clinical and experimental studies revealed that pioglitazone has favourable effects in various cardiovascular diseases [3] The investigation of the vascular effects of pioglitazone in an experimental T2D model may be important to identify a new mechanism of action related to PVAT.

In this project, T2D model is induced in wistar rats fed with high fat diet/low dose streptozotosin administration. [4] Thereafter, 20mg/kg pioglitazone is applied for 6 weeks. The effect of pioglitazone on vascular reactivity is evaluated on isolated thoracic aorta of T2D rats. In addition, blood and PVAT samples obtained at the end of experimentation are used for measurement of biochemical parameters (cholesterol & triglyceride) & adipokines. According to our findings, in this type-II diabetic model, pioglitazone increased body weight and reduced serum trigliseride levels. Pioglitazone increased PVAT amount but did not statistically significantly influence its anticontractile effect on vascular reactivity. Our findings are the first that displays the effects of pioglitazone on vascular function and biochemical parameters in high fat diet/low dose streptozotosin induced diabetes model.

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Short communication 20 (WG2)

The journey from ethnobotanical studies to pharmacological target: The effect of imperatorin and isoimperatorin on penile relaxation.

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Background: Ethnobotanical studies show that *prangos* roots are traditionally used as an aphrodisiac in Anatolia (1). Thus we had tested the extract of the roots of P. Pabularia in our previous studies, which is an endemic species of prangos (Umbelliferae) in Turkey. Because we found that *P. pabularia* extract increases erectile function, we further aimed to investigate the effect of its major compounds in the relaxation of penile tissue. We found that oxypeucedanin (oxy, %9.96), imperatorin (imp, %5.2) and isoimperatorin (isoimp, %9.26) are the major compounds in the chloroform extract of the roots of *P. pabularia* by HPLC analysis.

Methods: A new gasotransmitter hydrogen sulfide (H_2S) has been shown to play a role in erectile function (2). Erectile function of mice penile tissue was evaluated by vasorelaxation response measured by DMT strip myograph. Aminooxyacetic acid; (AOAA, 10mM, 30 min) which is a general inhibitor of H_2S synthesis was used to investigate the role of H_2S.

Results: We found that imp (10^{-7}-10^{-4} g/mL) and isoimp (10^{-7}-10^{-4} g/mL) caused relaxation in mice corpus cavernosum precontracted with phenylephrine ($3x10^{-5}$) compared to vehicle (E_{max}: %78.66±4.34 vs %27.87±4.47 and E_{max}: %81.40±3.59 vs %27.87±4.47 respectively, P<0.001, unpaired student t-test), Since H_2S plays an important role in penile relaxation (2), we further tested if H_2S has a role in these effects. Imp or isoimp induced vasodilatations were significantly inhibited by AOAA, (E_{max}: %78.66±4.34 vs %33.82±2.54 and E_{max}: %81.40±3.59 vs %43.24±3.77 respectively, P<0.001, unpaired student t-test, imp and isoimp with or without AOAA), suggesting H_2S formation can be the pharmacological mechanism of the imp and isoimp induced penile relaxation. We also examined effect of isoimp (100 µM, 30 minutes) on L-cysteine (L-cyst; 10 mM) stimulated H_2S formation in the presence or absence of AOAA (10 mM, after isoimp treatment) in mouse penile homogenates by methylen blue assay. Isoimp caused a significant increase in H_2S formation induced by L-cysteine (P<0.01, unpaired t test) and AOAA can reverse it in penile tissue homogenate (P<0.05, unpaired t test).

Conclusion: We conclude that imp and isoimp have a potential for the treatment of erectile dysfunction, by causing strong vasorelaxation in penile tissue. Further our result demonstrated that isoimp has a capability to induce L-cysteine induced H_2S formation. Since H_2S has several physiological and pharmacological effects, our result may lead further studies to investigate the effect of imp and isoimp in other pathological conditions where H_2S is beneficial, such as myocardial infarction, diabetes, and hypertension.

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Short communication 21 (WG2)

A potential repositioning of Trimetazidine for the treatment of bladder dysfunction: effects on mouse detrusor contractility and cytoprotection

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Trimetazidine (TMZ) is a novel anti-ischemic agent. This effect is mediated via inhibition of fatty acid oxidation and improvement of myocardial energy metabolism (1). Both metabolic and cytoprotective effects of TMZ in cardiac tissue has been extensively studied, however, its effects on other muscle tissue and potential therapeutic utility is unknown (2). The aim of our project is to critically evaluate the actions of TMZ on mouse bladder contractility and mouse model of cyclophosphamide (CP) - induced inflammation. To investigate the effect of TMZ on contractility, detrusor smooth muscle strips were obtained from male Balb/c mice (25-35 grams), and changes in isometric tension was recorded. TMZ pretreatment (300-1000 µM) attenuated carbachol- and KCl- induced contractions ($p<0.05$). TMZ (10-1000 µM) induced a concentration-dependent relaxation in KCl-pre-contracted strips (E_{max}=66.50±3.48). Incubation of detrusor strips with $BaCl_2$ (K_{ir} channel blocker) significantly decreased TMZ-induced relaxation whereas incubation of tetraethylammonium, glibenclamide, and 4-aminopyridine had no effect. TMZ pretreatment (300-1000 µM) significantly inhibited $CaCl_2$-induced contraction in Ca^{2+}-free Krebs solution, also reduced the contractile response to carbachol in the presence of nifedipine. To investigate the cytoprotective effects of TMZ, hemorrhagic cystitis was induced with CP (300 mg/kg, ip) and TMZ (10 and 20 mg/kg/day, ip; treatment) or vehicle (control) was applied for 3 consecutive days before CP. TMZ (20 mg/kg) prevented CP-induced histopathologic alterations. Collectively, our results demonstrate that TMZ inhibits detrusor contractility via affecting intracellular calcium release and by inhibiting K_{ir} channels. Non-vascular smooth muscle relaxation and cytoprotective effect of TMZ suggest that this novel molecule has a potential for repurposing/repositioning for bladder dysfunction and diseases.

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Short communication 22 (WG1)

A Lab-on-a-Molecule with an Enhanced Fluorescent Readout on Detection of Three Chemical Species

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We have designed and synthesised the first naphthalimide-based lab-on-a-molecule **1** (Figure 1).[1] A lab-on-a-molecule is a molecular device that simultaneously detects for a congregation of three (or more) biologically relevant chemical species.[2,3] The design concept consists of ferrocene as an electron donor responsive to the oxidant Fe^{3+}, piperazine as a receptor for binding H^+, and *N*-(2-methoxyphenyl)aza-15-crown-5 ether as a receptor for binding Na^+. Molecule **1** is unique as it incorporates three different titrimetric methods: H^+ by acid-base chemistry, Na^+ by complexation and Fe^{3+} by redox chemistry. In the presence of high threshold concentration levels of all three analytes, a bright green fluorescence is observed with the naked eye; however, in the absence of just one of the analytes no fluorescence is observed. This latest example exhibits the greatest fluorescence switching ratio and fluorescence quantum yield in aqueous methanol to date. Disease screening is a foreseeable application as specifically designed molecules could be used to test for key analyte combinations in a single rapid test and perform a diagnosis autonomously. A presentation aim is to give inspiration to those developing multi-target ligands.

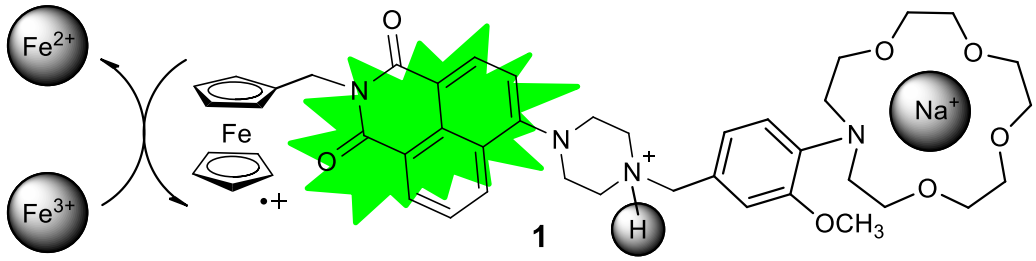

Figure 1: The molecule detects a congregation of three cations in aqueous methanol by fluorescence.

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Short communication 23 (WG3)

New screening libraries arising from the Prestwick Chemical Drug Library ®

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Developing a brand-new drug takes an enormous amount of time, money and efforts. However, there is a wide consensus that new drugs in many therapeutic areas are urgently needed meaning that it is crucial to advance strategies to reduce time frame, decrease costs and improve success rates.

Sir James Black, winner of the 1988 Nobel Prize in Physiology and Medicine, famously stated that: "the most fruitful basis for the discovery of a new drug is to start with an old drug". Disillusioned with HTS and struggling to bring new chemical entities to market, many labs are turning back to Sir James' wisdom. In this perspective, a range of valuable tools based on marketed drugs have been developed at Prestwick to support strategies such as:

1. Drug repurposing [1] via the Prestwick Chemical Library ® 
2. Fragment-Based Drug Discovery (FBDD) [2] via the Prestwick Fragment Library 
3. Using an innovative screening library arising from the combination of fragments of approved drugs

The design, properties and advantages of the Prestwick tools are presented and discussed in the present poster.

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Short communication 24 (WG3)

Lead to Target: A Computational Approach to Identify Possible Protein Targets of Molecules with Known Experimental Biological Activity

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A central problem in medicinal chemistry is the identification of the protein targets to which a specific molecule binds or interacts. Every year thousands of new molecules are tested experimentally for potential biological activity, particularly antimicrobial or anti-cancer. These molecules result from the continuous research efforts in organic synthesis on specific classes of compounds from many organic chemistry groups, from studies on plants leading to the identification of new phytochemicals, from compounds identified in the marine environment, and from new molecules obtained from other natural sources. Their activity is tested against specific organisms, cell lines, or other experimental models. Through these studies, some (if not many) of these molecules come to exhibit promising biological activity that justifies further study and investment. However, a major bottleneck comes at this stage. For these molecules to be improved, in a rational and effective way, knowledge on the specific target (enzyme, receptor, protein, etc) on which they act at the molecular level is required.

While some in vivo or in vitro strategies can be used to narrow down the list of possible protein targets, it is often a guess and test game that typically relies on the similarity with previous known compounds or intuition. Bioinformatics, computational biology and computational chemistry are disciplines that are growing in size and potentiality at an impressive rate, with borders that touch and intertwine, resulting in a large and continuous body of knowledge that is starting to fill, spanning from the organism, to the cellular, molecular, atomic and electronic level.

Here, we describe a multi-disciplinary computational approach that we have been developing at BioSIM in collaboration with different experimental groups, to identify the potential binding targets of specific molecules with confirmed experimental activity. For that we combine the information from large bioinformatics databases covering the full proteome of specific organisms of interest or different cell-lines, with the structural information obtained from the Protein Data Bank, molecular dynamics, inverse virtual screening, protein-ligand docking, molecular dynamics, free energy calculations and Quantum Mechanics/Molecular Mechanism Methods.

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Short communication 25 (WG4)

Drug design in silico: dream, (next) future or even coming reality?

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Atomic simulation is paramount at the very catalitic or allosteric site of a reactitve molecule, in order to try to understand every relevant detail, but then, getting away, a coarse understating of what molecular domains are doing is often more than sufficient [1]. Moreover, deduction from theory could very interestingly complement with understandable inference [2] from experimental evidence data, in order to get insight from the known effects of the implemented causes. Fusing together several approaches, carefully picking the best of each, one can hope to be more accurate and less sensible to noise than with just the deemed best approach. When dynamic data are available, like kinetic metabolism, a piecewise affine approximation [3], within the framework of hybrid modeling through dynamical and logical systems of observed process, allows to resort to principal component analysis in every almost linear identified interval, allowing to simplify relationships among really salient variables as in outperforming famous key genes identification in differential diagnosis [4] or discovering new pathways [5] discriminating every patient in known diseases. like leukemia, yielding to true personalized precision medicine when proper drug and proper posology could be better identified accordingly.

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Short communication 26 (WG4)

Computational Approaches in Multi-target Drug discovery

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During the last decades drug discovery process has been revolutionized and became more rational using approaches focused on the prediction of protein targets of small molecules and/or on the identification of potential hits. In most pharmaceutical companies and also in academia, beyond classical approaches, drug designers are taking advantage of using computer-assisted drug design (CADD) to identify novel bioactive molecules and/or select the best candidates for synthesis that simultaneously are likely to display the desired ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. The use of computational methodologies, ligand and/or structure-based, have been already proved as a synergistic tool to boost the drug discovery process. The traditional concept of one drug one target is evolving and multi-target drugs have come out as a promising option to combination therapies to overcome drug resistance. Also, the tremendous growth of databases with bioactivities and computational tools to predict protein targets of small molecules has been gaining importance in recent years. In this talk I'll briefly introduce some computational methodologies used to identify new hit compounds as an approach to find novel therapeutic agents and the application of databases to predict protein targets of a small molecule and to design multi-target ligands (1,2).

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Short communication 27 (WG4)

Multi-Target Ligand Design is Ripe for New Methods

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The concept of multi-target ligand design (MTDL) is expanding and giving fruits due to the awareness of the multiple targets and off-target effects of ligands and drugs.[1-7] In addition to new targets, such as newly discovered kinases [5], as well as oxadiazoles and indolizine-containing compounds for anti-leishmanial drug design[5], the effects of anti-targets [4], as well as multiple structure on targets [6] can help improve the downstream profile of designed compounds. New methods are becoming available such as t-SNE and Boosting [1], as well as data bias and unbalance detection in machine learning [1,2]. The statistical distribution of the first significant digits of the important profiles of log*P* (membrane permeability and association), log*S* (solubility, availability, and required dose), as well as p*K*a (distribution, absorption) for drugs and other compounds can be assessed and tested statistically for machine learning and data methods, in addition to leading the design of compounds suited for a selection of targets and off-targets. Each target and combinations of targets have their unique chemical space and the data, both calculated and experimental, can best reproduce the natural phenomena according to Benford's law.

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Short communication 28 (WG3)

New inhibitors of the BmrA pump identified through virtual screening

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Drug efflux mediated antimicrobial antibiotic resistance is a worldwide health problem. The current work focuses on new inhibitors of the ATP binding cassette drug efflux pump BmrA of *Bacillus subtilis*. This homodimer of 575 residues in each chain shares high similarity to the eukaryotic pump P-glycoprotein (P-gp).[1] Known P-gp inhibitors target either the nucleotide binding domain (NBD) or the groove between the two halves. However, since the angle between the two halves influences inhibitor binding, we focused only on the NBDs. MuTaLig-generated chemical library Chemotheca[2] was screened for the best binders. Modeling of BmrA was based on two homologs, the *Staphylococcus aureus* SAV1866 and the *Escherichia coli* EcMsbA. Taking EcMsbA with full coordinates (kindly provided by Prof. Geoffrey Chang) as the template and SAV1866 (PDB code: 2HYD) for the secondary structure information, Modeller 9.19 was used. The model was assessed with ProCheck, ProSa, and Verify3D for suitability. Virtual screening was performed with AutoDock Vina 1.1.2.[3] Binding energies were estimated for NBDs. Two criteria were set for inhibitor selection; (a) the average binding energy of a molecule to the NBD should be < -8.75, (b) the difference in the binding energy of a molecule to NBD of the first and the second chains should be $-0.45 <$ and < 0.45. Then molecules were screened for their ADME properties based on Lipinski's rule of five. This has left 12 inhibitor candidates. Among these, we were able to test only 5 molecules. The potential of these inhibitors was assayed monitoring ethidium bromide accumulation on 96-well plates using a Synergy HTX Multi-Mode Reader equipped with filters of 540 and 590 nm for excitation and emission, respectively. Among the tested molecules, CM13 and CM311 have shown potential. The authors acknowledge Marmara University, Scientific Research Projects Committee (FEN-B-129917-0534) and Mu.Ta.Lig COST ACTION CA15135 for support.

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Poster communications

Poster communication 1 – WG 1

Discovery of potent correctors of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) : a long way from photoactivation

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Of the about 2000 known CF mutations, deletion of phenylalanine at position 508 (F508del) in the CFTR protein is the most common one.[1] At present two small molecules targeting mutant CFTR are commercially available for CF patients: the corrector VX-809 and the potentiator VX-770 combined in a drug named Orkambi.[2] Correctors specifically address the folding and trafficking defects of F508del-CFTR protein. There is a general belief that treatment with a single corrector is not enough to achieve a clinically-relevant rescue of F508del defect and that a combination of correctors having complementary mechanisms is desired.[3] Linear and angular furocoumarins, studied for decades as photosensitizers (PSs), have been proposed in the last years as multitarget agents for the treatment of Cystic fibrosis (CF). We started our study from a library of about 200 compounds developed in our laboratory as photosensitizers[6] aiming at finding new chemotypes to maximize the rescue of mutant CFTR. Compounds were tested at the Telethon Institute of Genetics and Medicine (TIGEM) from which PP7 was initially identified and further improved (PP8). PP8 showed an interesting ability to functionally rescue F508del-CFTR, particularly in combination with VX-809. Importantly, it was also active in primary bronchial epithelial cells producing a marked synergic effect on transepithelial chloride secretion. Supported by our experience in the chemistry of nitrogen heterocycles, we synthetized 140 new molecules and, inspired by the pharmacological insight on the basis of structure-activity relationship (SAR). All compounds were tested as correctors using the halide-sensitive yellow fluorescent protein (HS-YFP) assay on CFBE41o- cells. Several new analogues demonstrated improved activity and PP28 stood out for its potency and efficacy compared to PP8. PP28 showed higher activity than VX-809 and a remarkable synergistic effect when used in combination with it corresponding to approximately 60% recovery of the normal CFTR functionality. For the relevance of these results, an international patent application with Telethon Foundation is ongoing.

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Poster communication 2 – WG 1

[1,2]oxazole[5,4-*e*]isoindoles: new derivatives and biological aspects

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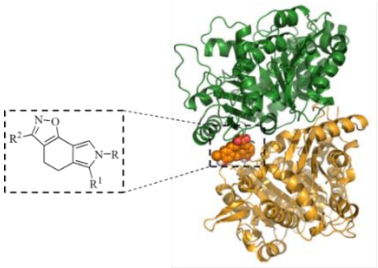
Tubulin-binding molecules represent an important class of antineoplastic agents. The therapeutic potential of antimicrotubule agents has been extensively exploited in clinical practice.[1,2] Recently, combretastatin A-4 (CA-4) has been considered a promising lead compound for the design and synthesis of novel microtubule-targeting agents.[3,4] CA-4 analogues containing the [1,2]oxazole ring as a linker of the two heterocyclic moieties displayed higher antitubulin activity than CA-4.[5] The [1,2]oxazolo[5,4-*e*]isoindole system, previously investigated by us, gave excellent results in preclinical studies reducing *in vitro* cell growth, impairing cell cycle progression and inducing apoptosis, as a consequence of the inhibition of tubulin polymerization, in experimental models of diffuse malignant peritoneal mesothelioma (DMPM).[6,7] Moreover, selected derivatives showed significant *in vivo* antitumor activity at well-tolerated doses in a DMPM xenograft model.

To obtain compounds with better drug-like properties and structure-activity relationship (SAR) informations, structural changes in the tricyclic core were further investigated. In particular, introduction of aminoalkyl chains or amides from primary and secondary amines in specific positions of the tricyclic scaffold were explored. Some of the synthetized compounds, screened at the National Cancer Institute in Frederick MD, confirmed strong antiproliferative effects in the micromolar-submicromolar range against the full panel of 60 tumor cell lines. Additionally, all new [1,2]oxazoles were tested against different lymphoma cell lines at the Institute of Oncology Research (IOR). The effects of compounds on tubulin polymerization were examined. Results will be discussed.



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Poster communication 3 – WG 1

Exploring the multi-target performance of mitochondriotropic antioxidants against the pivotal Alzheimer's disease pathophysiological hallmarks

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Alzheimer disease (AD) is the most common neurodegenerative disease featuring progressive and degenerative neurological impairments resulting in memory loss and cognitive decline. The specific mechanisms underlying AD are still poorly understood, but it is suggested that a deficiency in the brain neurotransmitter acetylcholine, deposition of insoluble aggregates of fibrillar β-amyloid 1-42 ($A\beta_{42}$), iron and glutamate accumulation play an important role in the disease progress. Despite the existence of approved cholinergic drugs, none of them demonstrated effectiveness to modify disease progression. Accordingly, the development of new chemical entities acting in more than one target is attracting progressively more attention as they can tackle intricate network targets and modulate effects.

In this work, a series of mitochondriotropic antioxidants inspired on hydroxycinnamic (HCA's) scaffold were synthesized, screened toward cholinesterases and evaluated as neuroprotectors in a differentiated human SH-SY5Y cell line. From the series, compounds **7** and **11** with a 10-carbon chain can be looked as multi-target leads for the treatment of AD, as they act as dual and bifunctional cholinesterase inhibitors and prevent the neuronal damage caused by diverse aggressors related with protein misfolded and aggregation, iron accumulation and excitotoxicity. The results obtained so far will be presented in this communication.

This project was supported by FEDER funds through the Operational Programme Competitiveness Factors -COMPETE and national funds by FCT – Foundation for Science and Technology under research grants (UID/QUI/00081, NORTE-01-0145-FEDER-000028, PTDC/DTP-FTO/2433/2014, PTDC/BIA-MOL/28607/2017, POCI-01-0145-FEDER-028607). S. Benfeito and C. Fernandes grants are supported by FCT, POPH and QREN.

Poster communication 4 – WG 1

Design and development of piperine derivatives acting as potent MAO-B inhibitors and mild P-gp inhibitors

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Parkinson's disease (PD) is a multifactorial age-related disorder clinically characterized by motor dysfunction[1], which has been associated to the loss of dopaminergic neurons in *Substantia Nigra* and consequent nigrostriatal dopamine deficiency [2]. Dopamine is deaminated into the corresponding aldehyde and hydrogen peroxide by two isoforms of monoamine oxidase (MAO), MAO-A and MAO-B [3], although it is mainly metabolized by MAO-B in *Substantia Nigra* [4]. The use of MAO-B inhibitors is therefore a strategy used to block dopamine degradation in the nigrostriatal pathway [2] and to prevent the formation of potentially toxic products [3].

The drug delivery into the brain is strongly hampered by the blood-brain barrier (BBB), which is partially due to the presence of P-glycoprotein (P-gp) in the luminal surface of the brain vascular endothelium [5]. P-glycoprotein can extrude a wide variety of structurally unrelated drugs (*e.g.* antiparkinsonian agents) and limit their access to the brain [6]. Therefore, a mild and reversible P-gp inhibition could enable the delivery of P-gp substrates to the brain [5].

As part of our drug discovery program, we developed a small piperine-based library (**Fig. 1**) aimed to find out new molecules able to act as potent MAO-B inhibitors and mild P-gp inhibitors.

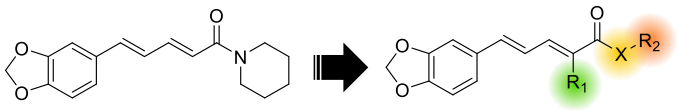

Figure 1: Chemical structure of piperine and the modifications introduced.

Fifteen piperine derivatives were successfully obtained. The *h*MAO inhibitory activities and the *h*MAO-B inhibition mechanism were evaluated. Cell-based assays were then performed to assess the cytotoxicity of the most promising compounds and their effect on P-gp transport activity. The results obtained so far will be presented in this communication.

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Poster communication 5 – WG 1

Synthesis and Anti-Cancer Activity of Novel Benzofuranylindolylmaleimide (BfIM) Derivatives

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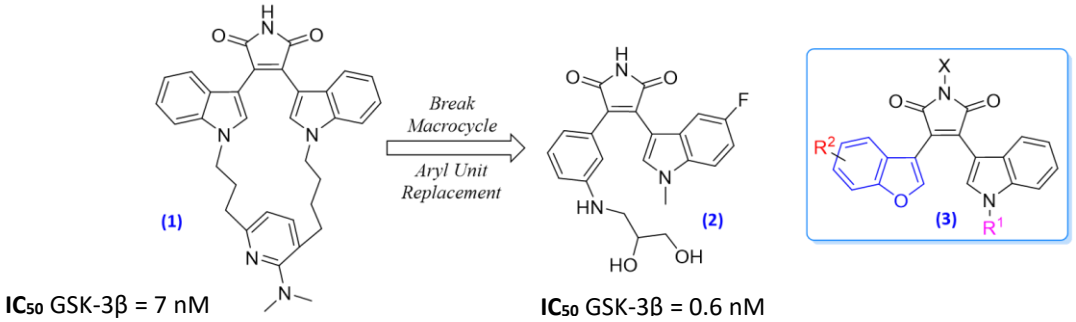
Protein kinases are a class of regulatory enzymes that are ubiquitous within the human body. They modify the function of proteins through phosphorylation of target substrates which have important downstream effects in intracellular signalling pathways.[1] One kinase of particular interest is glycogen synthase kinase-3 (GSK-3) as its overexpression has been implicated in cancer and a number of neurological disease states including bipolar disorder and Alzheimer's.[2] The bisindolylmaleimide (BIM) framework is the structural core of **(1)**, a nanomolar inhibitor of GSK-3β discovered by Johnson & Johnson, however, it is a potent inhibitor of many protein kinases.[3] Crystal structures have identified the importance of the maleimide ring of BIM which facilitate strong hydrogen bond interactions with the hinge of amino acids locking it place within the kinase pocket.[4] Improved binding affinity was achieved by replacement of one indole component for another aryl unit and preparation of **(2)** confirmed that both conformational change and additional H-bonding interaction were responsible for this specificity.[5] Other alternative BIM that incorporate the benzofuran moiety are less explored but GSK-3β inhibition has been reported. This aryl unit is of particular interest as it offers the opportunity to explore hydrophobic and solvent accessible space (SAS) surrounding the GSK-3β active site.[6]



IC_{50} GSK-3β = 7 nM

IC_{50} GSK-3β = 0.6 nM

Figure 1: Synthetic strategy towards BfIM series.

This work describes the versatile synthetic route towards the novel BfIM pharmacophore **(3)** by retaining a hydrogen bonding headgroup, altering substituents on the benzofuran component and carrying out a full assessment of steric probes. Novel candidates were tested using the National Cancer Institute 60 cell line screen exhibiting anticancer activity and molecular modelling was conducted on lead compounds.

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Poster communication 6 – WG 1

Estimation of gastrointestinal absorption of a series of dual DNA Gyrase and Topoisomerase IV inhibitors using Pampa technique

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In this study, estimation of gastrointestinal absorption of thirteen selected dual DNA gyrase and topoisomerase IV inhibitors was carried out using PAMPA test. Diffusion through artificial membrane, consisting of egg lecithin solution in dodecane (first PAMPA model) and a mixture of hexadecane and hexane (second PAMPA model), was monitored [1,2]. The starting solutions (pH 5.5) and the acceptor medium (pH 7.4) were prepared to contain 2% dimethyl sulfoxide. Concentrations of tested compounds in starting solutions, donor and acceptor medium after incubation were measured using LC-MS/MS method. Permeability coefficients were calculated and good correlation was observed between results obtained using these two PAMPA models. Subsequently, the hexadecane/hexane model was selected for the evaluation of gastrointestinal absorption of the remaining ten compounds.

Derivatives with the highest permeability in hexadecane/hexane model were TZS-34 and TCF-3a (logPe: -5.37 and -4.93, respectively) whereas TLK-13 and NZ-97 had the lowest permeability (logPe: -9.91 and -9.85, respectively). Therefore, the highest gastrointestinal absorption can be expected from TZS-34 and TCF-3a, and lowest from TLK-13 and NZ-97 (Figure 1). High membrane retention observed for compounds TEL-28 (72%) and TAZ-2b (30 %) might be a reason for lower permeability than expected based on their lipophilicity.

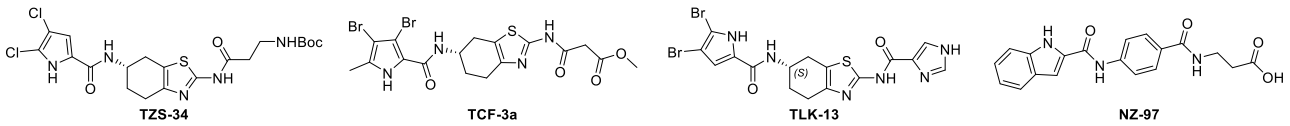

Figure 1: Chemical structures of underlined compounds.

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Poster communication 7 – WG 1

Discovery of potential Dual EZH2 and Proteasome Inhibitors as anticancer agents using computational methodologies

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Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase that is of great interest in human cancer.[1] EZH2 is frequently deregulated in cancer, and its overexpression was reported in solid malignancies such as prostate cancer and multiple myeloma (MM), also playing a key role in the development of drug resistance in MM. Until now, there is no cure for patients with MM, with the great majority of them developing resistance and/or relapse after the first-line therapy with new agents. Another important target in MM is proteasome and the first proteasome inhibitor, bortezomib, revolutionized MM treatment about 15 years ago.[2] Nowadays there are several combinations of proteasome inhibitors with other drugs have been approved by FDA, however, resistance remains an important drawback. Multitarget drugs have come out as a promising combination therapy option to overcome drug resistance.

The aim of this study was to identify and characterize computationally the first dual EZH2 and 20S proteasome inhibitors, as a promising alternative for the treatment of MM. To do that, bioactivity data for each protein was used in docking studies against both proteins. The best candidates showed significant resemblance with the co-crystal ligands in terms of their residues interactions profiles (figure 1). After that, the best molecules were chosen to create new derivatives datasets. Additionally, molecular dynamics simulations were carried out with the best candidate obtained from docking. The results will be presented and discussed.

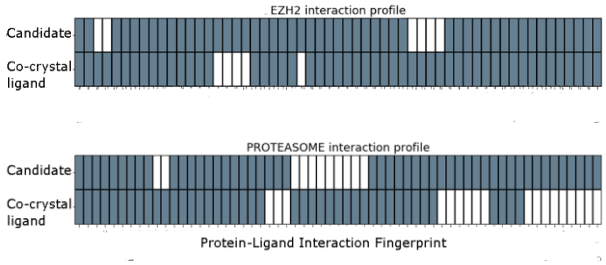

Figure 1: Protein-Ligand interaction fingerprints for a proteasome 20S (chymotrypsin-like site) and EZH2 candidate.

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Poster communication 8 – WG 1

Design and development of new antimicrobials based on the benzopyrone scaffold

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The chemical space of traditional single molecules hasn't been yet fully scoped and can be further exploited for the discovery and development of new chemical entities (NCE) with antibacterial activity, energising the antibiotic pipeline and addressing the existent clinical needs to fight the antimicrobial resistance.[1] In this context, we can look at heterocycles for inspiration, as benzopyrone nature's privileged scaffolds. The benzopyrone core, namely chromones and coumarins displays great structural variety and a wide range of pharmacological activities.[2-4] Furthermore, chromone and coumarin derivatives can be considered isosteric surrogates of quinolones, a scaffold widely present in antibiotics and several molecules with biological applicability.

Thus, the project herein presented aims the design and development of new antimicrobial compounds based on the benzopyrone scaffold of chromones. Accordingly, a small, diverse and concise chemical library of benzopyran-based derivatives was synthesised and screened against several microorganisms (5 bacteria and 2 fungi strains) to assess their antimicrobial profile. Moreover, minimum inhibitory concentration (MIC), cytotoxicity and haemolytic data were obtained for the best compounds of the series. The preliminary structure-activity relationship studies performed will guide further structural refinement and optimisation. 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/00081/2020, PTDC/ASP-PES/28397/2017).

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Poster communication 9 – WG 1

Iridium-catalyzed alkylation reactions for pharmaceutical industry

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Branched ketones, alcohols, alkylnitriles and quinolines are ubiquitous in natural products, pharmaceuticals and broadly used as valuable intermediates in multi-step organic synthesis. In general application of pre-functionalized alkyl halides with toxic bases are used for their synthesis, relieving stoichiometric waste. Therefore, development of green and sustainable methods for these products is in demand. In this direction, transition-metal catalyzed alkylation of secondary alcohols or ketones by using primary alcohols is an attractive approach for the synthesis of substituted branched products. Such processes follows hydrogen autotransfer (HA) or borrowing hydrogen (BH) approach for the construction of new C-C and C-N bonds and generate water as the sole byproduct.

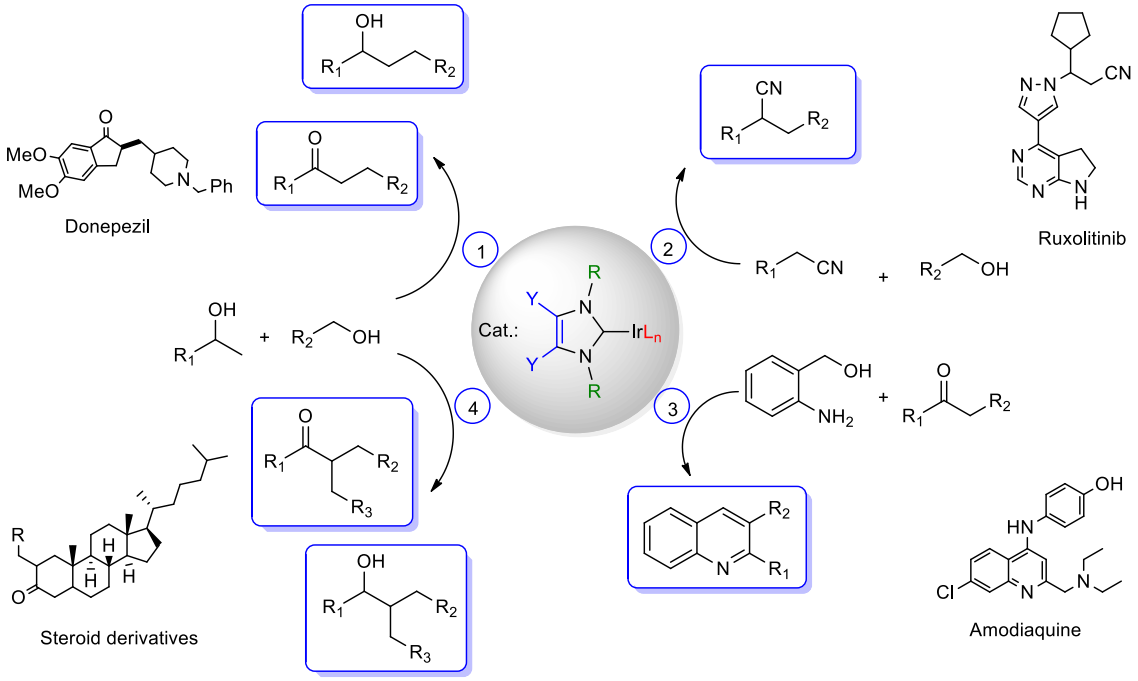

Figure 1: General representation of Ir-NHC catalyzed alkylation reactions.

In this presentation, we will cover in detail our latest results on the formation of C–C and C–N bonds *via* acceptorless dehydrogenative hydrogen autotransfer reactions using low amount of base and Ir-NHC complexes as catalyst.[1,2]

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Poster communication 10 – WG 1

Chemical modification of cotton fabric with 1,8-naphthalimide for its use as heterogeneous sensor and antibacterial textile

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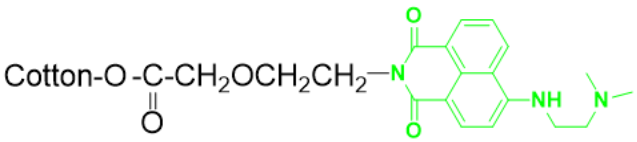
The basic photophysical characteristics of 4-(2-*N*,*N*-dimethylamino)ethylamino-N-(2-hydroxyethyl)-1,8-naphthalimide (NI) have been investigated in organic solvents of different polarity. The smart textile material has been obtained by modifying a cotton fabric containing chloroacetyl chloride and its subsequent dyeing with the NI.



Scheme: Modified with NI cotton fabric

Different quantities of the new functional acetylchloride groups have been introduced into cellulose macromolecules. The process involves chemical modification with chloroacetyl chloride by varying its concentration and the temperature of the treatment. The acetylation degree has been estimated by FTIR spectroscopy and weight gain method. After dyeing with NI the color of cotton fabric is yellow-green with a reflectance maximum at 530 nm and its hues depend on the degree of modification. The immobilized 1,8-naphthalimide on the textile matrix exhibits a sensitivity to Zn(II) ions better than that to Cu(II) ions. The antimicrobial activity of NI has been studied against Gram-positive and Gram-negative bacteria and the yeasts *Candida*. The results showed high antimicrobial activity against *B. cereus, A. johnsonii* and *C. lipolytica*. Chemically bonded NI to the cotton fabric has been found to reduce the growth of *B. cereus*, *A. johnsonii* and *P. aeruginosa.*

Poster communication 11 – WG 1

Synthesis and structural characterization of some chalcone derivatives

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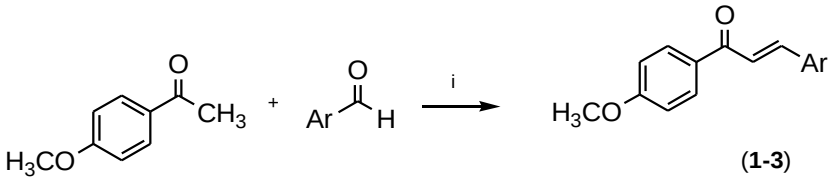
Chalcones are 1,3-diaryl-2-propenonea, and they are also known as α-β-unsaturated ketones (**1-3**, Scheme1). They are not only important precursors for synthetic manipulations but also form a major component of the natural products. Chalcones as well as their synthetic analogues display enormous number of biological activities such as antiinflammatory, antimicrobial, antifungal, antibacterial, antioxidant, cytotoxic, anticancer, antitubercular, carbonic anhydrase inhibitory activities [1-3].

In this study, synthesis of (E)-1-(4-methoxyphenyl)-3-aryl-prop-2-en-1-one were realised by Claisen-Schmidt condensation in basic condition. A mixture of 4-methoxyacetophenone (1 mmol) and a suitable aldehyde (1 mmol)[thiophene-2-carbaldehyde (**1**), 2-naphthaldehyde (**2**), benzo[d][1,3]dioxole-5-carbaldehyde (**3**)] was dissolved in ethanol (5 ml). Aqueous sodium hydroxide solution (10%, 10 ml) was added into the mixture under cold condition (0–5 °C). After overnight stirring at room temperature, the reaction mixture was poured into ice water mixture (50 ml) and acidified with HCl solution (10%) to pH = 6-7 (Scheme 1). The crude was filtered and crystallisation from ethanol. Chemical structures of the compunds were confirmed by ^{1}H NMR. In further studies, biological effects of these compounds on carbonic anhydrase isoenzymes will be tested.



(**1-3**)

i: NaOH (%10), ethanol, rt, Ar: thiophen-2-yl (**1**), naphthalen-2-yl (**2**), benzo[d][1,3]dioxol-5-yl) (**3**)

Scheme 1. The synthetic route for the synthesis of compounds **1-3**

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Poster communication 12 – WG 1

Multifunctional anti-Alzheimer's agents with anti-aggregating properties targeting neurotoxic Beta-amyloid peptide and Tau protein

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For searching of new bioactive molecules against complex Alzheimer's disease (AD) the multi-target-directed ligand design strategy has been applied. Our research focuses on multifunctional ligands that influence cholinesterases as a symptomatic target in the treatment of AD and amyloid beta (Aβ) and tau proteins as disease-modifying targets.[1,2] The recent studies led us to identification of some lead compounds with broad biological activity, including anti-cholinesterase and anti-beta secretase inhibitory potency, Aβ and tau anti-aggregation activity, neuroprotective effect against Aβ toxicity, and beneficial effects on memory *in vivo*. Among the series of 1-benzylamino-2-hydroxyalkyl derivatives, we discovered multi-target-directed ligands with very high anti-aggregating properties towards $A\beta_{42}$ (around 60% at 10 μM) and tau protein (in the range of 60 – 80% at 10 μM) *in cellulo* assay in *Escherichia coli*. These studies enable to identify a 3-*tert*-butylbenzylamino substituent responsible for both anti-aggregating activities. Among the series of 1-benzylpyrrolidine-3-amine derivatives, we identified multifunctional compounds with a balanced potency against butyrylcholinesterase and beta secretase, endowed with anti-aggregating properties against Aβ and tau protein as well as with antioxidant and metal-chelating properties. All these compounds showed dual anti-aggregating properties towards $A\beta_{42}$ (27.9 – 58.2% at 10 μM) and tau protein (52.8 – 79.5% at 10 μM). The results of the presented research will form the basis for further development of novel anti-AD agents.

Acknowledgments

This work was supported by the National Science Centre, Poland (grant UMO-2016/21/B/NZ7/01744).

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Poster communication 13 – WG 1

Multi-target profile of cashew nut-shell liquid (CNSL)-tacrine hybrids as a sustainable treatment for Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia and a global health problem: a big increase in longevity and the lack of treatments are the reasons of an ever-increasing number of people affected by the disease AD worldwide, particularly in the developing countries. Moreover, the cost of the drugs in use nowadays is too high and is important to provide affordable and globally accessible medications. [1] With these concepts in mind, we explored the potential use of cashew nut-shell liquid (CNSL), inedible by-product material, as a starting point to design multi-target hybrids with tacrine. CNLS derivates have innate multi-target mechanisms of action such as anti-inflammatory, antioxidant and hepatoprotective activity, while tacrine is a well know acetylcholinesterase inhibitor. [2]

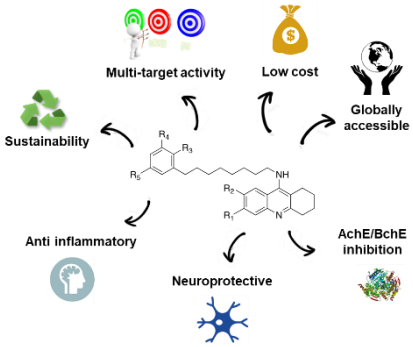

Figure 1: Advantages and multi-target profile of CNLS-tacrine hybrids

We synthesized a series of hybrids that were preliminary screened to test their hepato- and neurotoxicity. The non-toxic compounds were selected and showed neuroprotective and neuroinflammatory effects and one of them also showed remarkable inhibitory activity for butyrylcholinesterase (pM range).

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Poster communication 14 – WG 1

Synthesis of Furobenzopyrone Derivatives with Potential Anticancer Activity

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Carbonic anhydrases (CAs) are a class of metallo-enzymes that catalyze the reversible hydration of carbon dioxide into bicarbonate and a proton and are widely distributed in all living organisms [1,2]. These enzymes are involved in numerous physiological processes such as ion transport, regulation of pH, bone resorption, and secretion of gastric, cerebrospinal fluid and pancreatic juice [3]. In mammals CAs have 16 different isoforms and multiple ones implicated in a range of diseases, including cancer [2]. In particular, the trans-membrane CAs IX and XII are key pH regulators that create a differential pH microenvironment within solid tumors and allow for tumor cell survival under stressful conditions [2]. For this reason CAs became an increasing interest to researchers as drug targets, and, as a result, a number of CAs inhibitors have been designed [1]. However, the CAs inhibitors available are mostly unselectively leading to several side effects, thus selectivity is mandatory [3]. Coumarins and chromones are two groups of heterocyclic compounds commonly found in nature that show a wide range of biological activities, such as aromatase inhibitory effect, anti-HIV, antimycotic, and antitumor activities [4]. Previous results also highlighted the selectivity of furocoumarins towards CA IX and XII [5]. Accordingly, our project will focus its drug design strategy on heterocycle compounds based on the coumarin and chromone scaffolds and in the development of drug candidates that can inhibit selectively CA IX and XII. In **Figure 1** the two families proposed for synthesis, furochromones and furocoumarins, are reported. The results obtained so far will be presented in this communication.

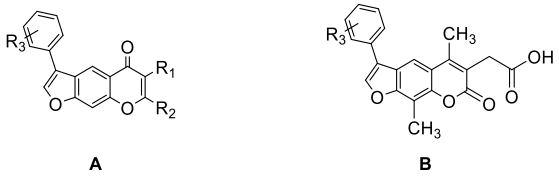

Figure 1: General structures of the compounds proposed for synthesis.

Acknowledgements:

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Poster communication 15 – WG 1

An atypical catechol *O*-methyltransferase inhibitor is a remarkable modulator of hypoxia in HeLa cells

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Catechol O-methyltransferase (COMT) inhibitors are valuable co-adjuvant drugs in the clinical management of Parkinson's disease [1]. Standard COMT inhibitors are based on the nitrocatechol scaffold, which chelates the Mg co-factor within COMT and lead to a tight-binding inhibition mechanism. While effective, these drugs have been linked to drug-induced hepatoxicity and poor brain bioavailability [2]. In our study, we explored alternative scaffolds that mimic the pharmacological behavior of nitrocatechols under physiological conditions and focused our strategy on heterocycle catechol mimetics [3]. This led to the discovery of TS22, a *N*-aryl substituted 3-hydroxypyridin-4-one that operated as a potent inhibitor of COMT in rat liver homogenates. The activity of TS22 was confirmed in MB-COMT and S-COMT from rat liver and brain, where we observed potent inhibition of the purified proteins in the low nanomolar range. We subsequently studied the effect of TS22 in different cell lines to evaluate its safety compared to standard COMT inhibitor tolcapone. TS22 did not induce any toxic effects in a panel of cell lines (SH-SY5Y, Caco-2, SU-DHL-10, WSU-DLCL2, VAL, U2932, SU-DHL-2), but we found a surprising effect in HeLa cells: TS22 led to a massive induction of hypoxia-inducible factor 1-α (HIF-1α). Increased HIF-1α was observed by immunoblotting when HeLa cells were incubated with TS22 100 µM both in normoxia and hypoxia. This was consistent with an upregulation of the hypoxia response element (HRE luciferase assay), which are regulated by HIF-1α. Due to its structural similarity to standard iron(III) chelators, we hypothesize that TS22 is able to stabilize HIF-1α by inhibiting protein-protein interactions with prolyl-4-hydroxylases (PHDs) that target HIF-1α for degradation. This stabilizing effect increases transcription of HIF-1α related survival genes. Together with its ability to inhibit COMT and prevent dopamine catabolism, the HIF-1α stabilizing effect of TS22 yields a promising dual-target outline, which may be of therapeutic interest for Parkinson's disease.

This project is supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2020, PTDC/MED-QUI/29164/2017 and SFRH/BPD/114945/2016)

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Poster communication 16 – WG 1

Irreversible Butyrylcholinesterase inhibitor/antioxidant hybrids show pronounced in vitro activities and in vivo neuroprotectivity

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Butyrylcholinesterase (BChE) has been validated as a target for Alzheimer's disease (AD) treatment because BChE knock-out mice show lowered vulnerability to amyloid-induced amnesia.[1] Also "chemical knock-out" by highly selective inhibitors show pronounced anti-neuroinflammatory and pro-cognitive effects.[2] Due to the fact that AD is associated with inflammation in the brain,[3] we have incorporated antioxidant moieties into a pseudo-irreversible BChE-inhibitor to obtain multi-target-directed ligands (MTDLs). The designed MTDLs show a similar antioxidant *in vitro* profile compared to the parent antioxidants (measured by DPPH, ORAC, and metal-chelating assays), while being slightly less potent on the BChE (three-digit nanomolar inhibition) compared to the parent BChE inhibitor. Furthermore, both potential neurotoxicity, as well as prevention of glutamate-induced intracellular formation of oxidative stress in a murine hippocampal cell line (HT-22) were investigated.

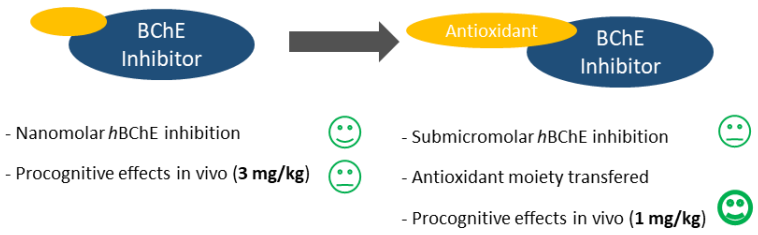

Figure: Design of pseudo-irreversible BChE-inhibitor MTDLs with antioxidant properties

The most promising compounds *in vitro* were tested in an established *in vivo* AD mice model.[1,2] Despite lower *in vitro* BChE inhibition, MTDLs tested *in vivo* showed a pronounced effect, even at a much lower dosage compared to the BChE inhibitor parent molecule. The data confirms synergistic effects of MTDL *in vivo* and supports the MTDL approach.

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Poster communication 17 – WG 1

Mitochondriotropic antioxidants as novel therapeutics for Amyotrophic lateral sclerosis: development of multitarget agents to prevent stress induced motor neurons death.

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Amyotrophic lateral sclerosis (ALS), also known as Gehrig's disease, is a devastating multifactorial neurodegenerative disease characterized by the progressive loss of motor neurons (MNs) in the brain stem, spinal cord and motor cortex.[1] The patients usually succumb to the disease 2-5 years after diagnosis experiencing severe muscle atrophy and ultimately death by respiratory failure. However, despite the severity of the disease the only drugs approved for the treatment of ALS (Riluzole and Edaravone) modestly extend the survival of the patients (average 3-4 months), without changing the fate of the disease.[2] Hence, there is a great pressure for the development of novel therapeutic solutions for ALS.

Oxidative stress has been associated with motor neuron degeneration and mitochondrial dysfunction, contributing decisively to ALS pathology.[3] Indeed, mitochondria dysfunction is observed early in ALS, suggesting mitochondria as valuable therapeutic target for ALS. Furthermore, the cross-talk between oxidative-stress/mitochondria dysfunction and two transcription factors with impaired activity in ALS, Nuclear factor- kappa B (NF-κB) and Hypoxia inducible factor (HIF), further validate this therapeutic approach.[4] In this context, our group proposes an innovative multi-target therapeutic approach for ALS based on mitochondriotropic antioxidants derived from benzoic acids present in human diet and, therefore capable to protect or minimize oxidative stress and inflammation driven MN death.

The mitochondriotropic antioxidants were capable to directly act in mitochondria preventing oxidative stress damage and inhibit caspase 3 activation on SH-SY5Y cells expressing mutSOD1 (ALS cellular model). In this communication are presented the most recent results obtained with our novel multi-target chemical entities.

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Poster communication 18 – WG 1

Discovery of Orexant and Anorexant Agents with Indazole Scaffold Endowed with Peripheral Antiedema Activity

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The Endocannabinoid system represents an integrated neuronal network involved in the control of several organism functions, such as feeding behavior. A series of hybrids of 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (Rimonabant), a well-known inverse agonist of cannabinoid receptor CB1, once used as an antiobesity drug, and the *N*-(2*S*)-substitutes of 1-[(4-fluorophenyl)methyl]indazole-3-carboxamide with 1-amino-3-methyl-1-oxobutane (AB-Fubinaca), 1-amino-3,3-dimethyl-1-oxobutane (ADB-Fubinaca), and 3-methylbutanoate (AMB-Fubinaca), endowed with potent agonistic activity towards cannabinoid receptors CB1 and CB2, were synthesized in solution as *C*-terminal amides, acids, methyl esters and *N*-methyl amides.[1] These compounds have been studied by binding assay to cannabinoid receptors and by functional receptor assay *in vitro*. The most active among them as agonist (S)-1-(2,4-dichlorobenzyl)-*N*-(3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-1H-indazole-3-carboxamide (**LONI11**) and antagonist (S)-2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)-3-methylbutanoic acid (**LONI4**) were tested *in vivo* to evaluate their ability to stimulate or suppress the feeding behavior after intraperitoneal (i.p.) administration. For **LONI11** formalin test and tail flick test after administration by the subcutaneous (s.c.) and intracerebroventricular (i.c.v.) routes respectively, were also carried out *in vivo* in mice to investigate the antinociceptive property at the central and peripheral level. We observed a significant orexant effect for **LONI11** and an intense anorexant effect for (S)-methyl 2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (**LONI2**) and **LONI4**. In Zymosan-induced edema and hyperalgesia, **LONI11** reduced the percent of paw volume increase and paw latency after s.c. administration, also suggesting a possible peripheral anti-inflammatory activity.

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Poster communication 19 – WG 1

4-phthalimido-*N,N*-diethylbenzensulfonamide as MTDL for the treatment of Alzheimer's Disease and Type II Diabetes Mellitus

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For the last few decades, research studies have provided the relationship between type II diabetes mellitus (T2DM) and Alzheimer's disease (AD). Besides, the development of one increases the risk of other. A variety of mechanisms has been postulated in the risk of these diseases such as metabolic abnormalities of insulin resistance (dyslipidemia, hypertension), hyperglycemia itself or insulin, by disturbing synaptic plasticity, learning and memory[1]. Nowadays a number of promising approaches to AD treatment have been formulated. One of the leading approaches in the drug design strategy relies on the synthesis of multi-target directed ligands (MTDLs) for the treatment of multi-factorial AD[2]. In an effort to develop new MTDLs for the treatment of both AD and T2DM, in our preliminary studies we design and synthesize 4-phthalimido-*N,N*-diethylbenzenesulfonamide derivative (Figure 1) and evaluate this promising candidate's inhibitory activity against AChE, BChE and α-glucosidase enzymes as potent MTDL, in which phthalimide and sulfonamide pharmacophoric group were combined.

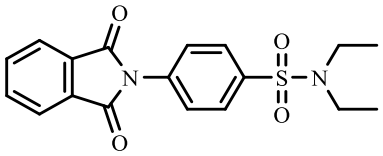

Figure 1: Structure of the synthesized compound as MTDL.

The synthesis of the title compound was realized in three steps according to the procedure in the literature[3]. The synthesized compound was screened for its *in vitro* cholinesterase inhibitory activity using slightly modified colorimetric method of Ellman et al. with galantamine. α-Glucosidase inhibitory activity was performed spectrophotometrically by using slightly modified method of Zawawi et al. Acarbose was used as a reference. In addition, molecular modeling study of the compound against AChE demonstrated binding interactions with both PAS and CAS of the enzyme. According to biological activity results, this sulfonamide derivative exhibited good to moderate inhibitory activity against the desired three enzymes: AChE (IC_{50} 1.35 ± 0.08 µM), BChE (IC_{50} 13.41 ± 062 µM) and α-glucosidase (62.5 %). The molecular modeling studies were showed its binding modes with the respective enzyme's active sites. It is believed that this derivative could be considered future candidate for further studies and open new doors for the researchers interested in the search for new MTDLs.

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Poster communication 20 – WG 2

CSE-H_2S pathway is an important target under inflammatory condition in the improvement of endothelial function

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Endothelium plays a key role in vascular hemostasis through regulating angiogenesis, inflammatory immune response and modulating vascular tone. Pro-inflammatory cytokines have been shown to impair endothelial-dependent vasodilation through reduced nitric oxide (NO) bioavailability and increased superoxide radical formation[1] Cystathionine γ-lyase (CSE) derived hydrogen sulphide (H_2S) is an endogenous gasotransmitter that plays important roles in many physiological functions, including vasodilatation, angiogenesis[2]. Mice lack of CSE are pro-atherogenic, hypertensive and exhibit enhanced myocardial ischemia–reperfusion injury due to increased lipid uptake, reduced endothelium-dependent vasodilation and enhanced susceptibility to oxidative stress[3]. So far, the relationship between inflammation and CSE/H_2S pathway is less clear. Therefore, we aim to investigate the role of CSE/H_2S pathway on endothelial function under inflammatory condition. CSE knockout and wild-type (WT) mice were used in this study. First and second order of mesenteric resistance arteries were isolated from 12-16 week old mice. Vascular functions were measured by wire myography system. Phenylephrine (PE) induced contractions were not found different between wild type and CSE knockout mice. Whereas, deletion of CSE impaired acethylcholine-induced endothelium dependent relaxations in first and second branches of mesenteric arteries. This impairment was more dramatic in second branches of mesenteric arteries, which are micro resistant arteries compared to first branches. The role of CSE pathway in endothelial function under inflammatory condition were evaluated by in vitro pretreatment of the arteries with proinflammatory cytokine TNF-α. Deletion of CSE increased sensitivity to TNF-α to exacerbate endothelial dysfunction. We conclude that, deletion of CSE sensitizes the arteries to proinflammatory cytokine, TNF-α, and exacerbate the TNF-α-induced endothelial dysfunction. Our results imply that CSE is important target to protect vascular system in many diseases where both endothelial dysfunction and inflammation play an important role in the pathogenesis.

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Poster communication 21 – WG 2

Aminoalcoholate-driven tetracopper(II) cores as inhibitors of aggregation of β-amyloid

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The major hallmark of Alzheimer's disease is the accumulation of β-amyloid (Aβ) in the brain indicating that targeting of Aβ is good strategy for therapeutic development. In our earlier study we identified aminoalcoholate-driven tetracopper(II) core compounds namely, $[O\subset Cu_4\{N(CH_2CH_2O)_3\}_4(BOH)_4][BF_4]_2$ (**1**), $[Cu_4(\mu_4\text{-}H_2edte)(\mu_5\text{-}H_2edte)(sal)_2]_n\cdot 7nH_2O$, ($H_4edte$ = N,N,N',N'-tetrakis(2-hydroxyethyl)ethylenediamine, H_2sal = salicylic acid) (**2**), and $[\{Cu_4(\mu_3\text{-}Hbes)_4(\mu\text{-}hba)\}K(H_2O)_3]_n$, H_3bes = N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (**3**), as a novel class of acetyl and butyrylcholinesterase inhibitors with IC_{50} values in low micromolar range [1].

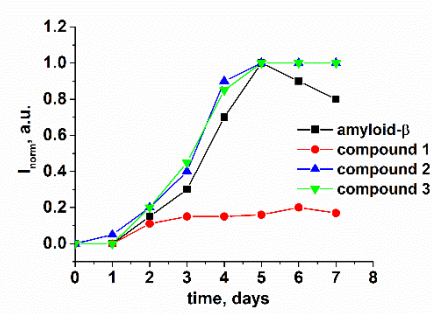

Figure 1: Fibrillation kinetics of Aβ1–40 in the absence and in the presence of compounds **1**,**2** and **3**.

In order to estimate multi target property of these compounds, we elucidated the effect of these compounds on the assembly of Aβ1–40 into amyloid fibrils, using in vitro thioflavin T (ThT) fluorescence assay. When fresh Aβ1–40 alone was incubated at 37°C, ThT fluorescence as a function of incubation time showed a sigmoidal shape (**Figure 1**). However, in the presence of **1**, ThT fluorescence did not increase, which indicated that Aβ formation was blocked. On the other hand, in the presence of **2** and **3**, changes in the fluorescence of ThT dye are not observed compared to control. Correlating this effect with structural or compositional features of investigated compounds, we concluded that the charge of the compounds might play a key role in Aβ recognition and amyloid inhibition indicating that positive charged compounds may act as multi target compounds for the treatment of Alzheimer's disease.

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Poster communication 22 – WG 2

WNT/β-catenin signaling: a new player in diabetic erectile dysfunction?

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Diabetes is a chronic metabolic disease with a dramatically increasing prevalence worldwide. Diabetes-induced erectile dysfunction (ED) is a common complication in diabetic men, leading to a significant decrease in the patients' quality of life, while current pharmacotherapy has limited efficacy and undesired side effects (1,2). Therefore, therapeutics that target novel cellular pathways and with improved efficacy and safety profile are needed for diabetic ED. Wnt/β-catenin signaling has been implicated in some diabetic complications, but the role of the Wnt/β-catenin signaling in diabetic ED is largely unknown (3,4). The aim of this study was to investigate whether Wnt/β-catenin signaling is impaired in a rat model of diabetic ED. Male Sprague–Dawley rats (8-10 weeks old) were randomly divided into diabetic and nondiabetic groups (n=6-7/group). Rats were injected intraperitoneally with streptozotocin (75 mg/kg) to induce diabetes. At week 12, erectile function was measured as cavernous nerve electrical stimulation-induced changes in intracavernous pressure, and penile tissue was collected for western blot analysis of Wnt/β-catenin signaling proteins including DKK1, phospho-GSK3β and active β-catenin. Erectile response was significantly reduced in diabetic rats compared to nondiabetic rats ($p<0.05$). Phospho-GSK3β and active β-catenin protein expressions in the penis were decreased in diabetic rats ($p<0.05$) without a significant alteration in DKK1 expression, demonstrating the decreased activation of the Wnt/β-catenin signaling in diabetes. This is the first report of dysregulated Wnt/β-catenin signaling in diabetic penile tissue leading to ED. Findings of this study, will prompt to investigate further role of Wnt/β-catenin signaling and the therapeutic potential of compounds that target this cellular pathway in diabetic ED.

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Poster communication 23 – WG 2

Role of TLR2/TLR4 signaling on cavernous nerve injury- induced erectile dysfunction

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Neurogenic erectile dysfunction (ED), a prevalent complication after radical prostatectomy, impairs the erectile capacity that leads to a significant decrease in patients' quality of life (1). Despite the extensive research, mechanism(s) of ED is poorly understood. Toll like receptors (TLRs) are important in innate immune response, neuroinflammation, and neuropathies although their contribution in neurogenic ED has not been examined (2,3). This study investigated the role of TLR2 and 4-mediated signaling in a rat model of bilateral cavernous nerve injury (BCNI)-induced ED. Male rats (275-400g) underwent BCNI or sham injury and randomized to 1, 3 or 7-days post-injury groups (n=5-6/group). Cavernous nerve electrical stimulation-induced erectile function was evaluated as maximum intracavernous pressure (mICP) and total ICP while continuously monitoring mean arterial pressure (MAP). Protein expression levels of TLRs signaling in penile tissue and major pelvic ganglia (MPG) were examined by western blot. mICP/MAP was lower in BCNI groups compared with sham groups at 1, 3 and 7-days whereas total ICP/MAP was lower in only 3 and 7-days post-injury. In the penile tissue, there was no change in TLR4 protein expression, but TLR2 expression was significantly increased at 7-days post-injury. In addition, MyD88, a downstream adaptor molecule of TLR, was lower at 3-days post-injury ($p<0.05$). In MPG, TLR2 and 4 expressions were reduced at 3-days ($p<0.05$), and MyD88 expression was significantly increased at 7-days post-injury. These results indicate that TLR pathway is involved in the pathophysiology of neurogenic ED. Our laboratory is currently investigating the further relation of the TLR pathway with fibrotic and apoptotic processes in penile tissue and penile innervation. This work was supported by the Scientific and Technological Research Council of Turkey, TUBITAK (project number 217S434).

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Poster communication 24 – WG 2

The Effect of Hyperforin which is the Specific Activator of the TRPC6 on Proliferation Rate and SOCE in Huh-7 Human Hepatocellular Carcinoma Cells

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Objectives: The purpose of our study was to investigate the potential effects of hyperforin which is the specific activator of the transient receptor potential canonical 6 (TRPC6) channel on proliferation rate, store-operated calcium entry (SOCE) and expression of SOCE-related proteins (TRPC1, TRPC6, STIM1 and Orai1) in Huh-7 human hepatocellular carcinoma cell line.

Methods: In this study, real-time quantitative PCR (qRT-PCR, Light Cycler 480, Roche) analysis was performed to investigate the effects of hyperforin incubation (24 h) on mRNA expression levels of TRPC1, TRPC6, STIM1 and Orai1. In order to investigate the effects of hyperforin on SOCE, changes in intracellular calcium concentrations in Fura-2-loaded Huh-7 cells were monitored on a spectroflurometer in real time. In addition, the effects of hyperforin on the proliferation of Huh-7 cells were examined using the real-time cell analysis system (xCELLigence).

Results: According to qRT-PCR results, hyperforin induced statistically significant increase in TRPC1 mRNA expression levels while STIM1 and Orai1 levels were decreased. On the other hand, although not statistically significant there was a significant increase in TRPC6 mRNA expression levels. CPA-induced SOCE was significantly increased in Huh-7 cells incubated with hyperforin (2 h) compared to control cells. At the same time, hyperforin application as alone or after CPA-mediated SOCE caused an increase in intracellular calcium levels. In order to investigate the role of protein kinase C in calcium concentration increases caused by hyperforin, in the presence of the PKC inhibitor Chelerythrine, hyperforin-induced $[Ca^{+2}]_i$ increasing was significantly reduced. Hyperforin caused a significant concentration dependent inhibition in the rate of proliferation of Huh-7 cells.

Conclusions: Hyperforin-induced potentiation of SOCE may be result of increases in mRNA expression levels of TRPC1 and TRPC6. Inhibitor effects of hyperforin on Huh-7 cells suggest us that hyperforin may be a potential drug for cancer treatment. Furthermore, hyperforin may play an important role in activation and regulation of SOCE in hepatocellular carcinoma cells.

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Poster communication 25 – WG 2

The search for Monoamine Oxidase B inhibitors among analogues of the potent histamine H_3 receptor ligand DL76

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Sometime ago we have described 1-(3-(4-*tert*-butylphenoxy)propyl)piperidine, named **DL76**.[1] This compound showed high affinity for human H_3R (hH_3R) *in vitro* (K_i = 22 nM) and *in vivo* (ED_{50} = 2.8 ± 0.2 mg/kg; p.o. mice). Moreover, **DL76** showed antinociceptive and antipruritic effects in acute, inflammatory and neuropathic pain.[2] Preliminary screening of our library of histamine H_3 receptor (H_3R) ligands for inhibitory activity towards human monoamine oxidase B (hMAO B) showed high inhibition of this enzyme by **DL76** (IC_{50} = 48 nM). Encouraged by these results we have designed two groups of DL76 analogues: (I) congeners with different amine moieties and (II) variants with a different substituent instead of the *tert*-butyl moiety. All compounds were evaluated in a radioligand binding assay with [^{3}H]*N*-methylhistamine as radioligand in HEK-293T cells, stably expressing the human H_3R and showed, with one exception, K_i values below 400 nM. The inhibitory activity towards hMAO B was evaluated using a fluorometric Amplex-Red assay. Thereby, most of the compounds displayed activity in the *submicromolar range*, with 1-(3-(4-*tert*-butylphenoxy)propyl)pyrrolidine (**E286**) ahead that exhibited hMAO B inhibitory activity with an IC_{50} of 2.7 nM.

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Poster communication 26 – WG 2

Microsomal PGE synthase-1 inhibitor reduces vascular tone and inflammation in human coronary bypass graft vessels

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Introduction: Human internal mammary arteries (IMA) and saphenous veins (SV) are frequently used for coronary artery bypass graft surgery. During bypass surgery, grafts are submitted to inflammatory conditions resulting in an increase of prostaglandin E_2 (PGE_2) synthesis[1-3]. Inhibition of this synthesis by COX-2 (cyclooxygenase-2) inhibitors like COXIBs is effective in reducing inflammation but their cardiovascular side effects limit their use[4]. Microsomal prostaglandin E synthase-1 (mPGES-1) is a terminal PGE_2 synthase in the COX pathway. Recently mPGES-1 inhibitors have been suggested as a potential novel therapy, an alternative to COX-2 inhibitors especially in the treatment of inflammatory diseases [2]. The aim of our study was to investigate the effect of mPGES-1 inhibitor on vascular tone and inflammation in IMA and SV.

Methods: Using an organ bath system, a first concentration-response curve induced by norepinephrine (NE) was performed on isolated human vessels: fresh or cultured (18h) in the presence or absence of both interleukin-1beta (IL-1β) and lipopolysaccharide (LPS). In addition, a second NE concentration-response curve was established after incubation 30 min with a mPGES1 inhibitor, Compound-3 (C3; 10 µM)[6]. Asymmetric dimethylarginine (ADMA), 6-keto-$PGF_{1\alpha}$, major metabolite of PGI_2, and PGE_2 release from human vessels were measured by ELISA.

Results: Under inflammatory conditions, the contractile responses induced by NE decreased in human SV or IMA. In these vascular preparations, incubation with C3 significantly attenuated the maximal contractions induced by NE (IMA -27±09%; SV -40±07%). These reductions were at least completely reversed after co-incubation with both C3 and IP (prostacyclin, PGI_2 receptor) antagonist Cay10441 (1 µM, 30 min). On the other hand, both 6-keto-$PGF_{1\alpha}$, PGE_2 and ADMA release were found significantly greater in SV versus IMA. The release of these mediators was significantly increased under inflammatory conditions. Moreover, incubation with C3 significantly reduced the release of PGE_2 and increased the release of 6-keto- $PGF_{1\alpha}$ from human SV and IMA.

Conclusion: The reduction in vascular tone and inflammation induced by mPGES-1 inhibitor is associated with an increased endogenous PGI_2 production and decreased PGE_2 synthesis, respectively. The treatment with mPGES-1 inhibitor might be beneficial to counteract graft vasospasm and vascular inflammation and therefore could increase patency rate of coronary graft vessels.

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Poster communication 27 – WG 2

Toluquinol derivatives as new multitargeted antiangiogenic drugs

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Toluquinol, a methylhydroquinone produced by a marine fungus, has already been described by us as a potent inhibitor of angiogenesis, lymphangiogenesis and tumor growth, suggesting a potential application of this compound in the chemoprevention and treatment of cancer and metastasis[1,2]. More recently we have synthesized a series of new toluquinol analogues and evaluated their cytotoxic activities, evidencing that the addition of new substituents may modulate the interaction of the toluquinol molecule and its targets, either changing the cytotoxicity or the antitumor selectivity of the new derivatives[3]. In this study, data regarding the capability of the different toluquinol derivatives to inhibit angiogenesis in vitro are presented, including their effects on different steps of the angiogenesis process, evaluated by means of a number of in vitro assays in vitro including proliferation, migration and tube formation. The mechanisms of action of the new compounds have also been studied, including their induction of endothelial cell apoptosis. Our results indicate that structural modifications of toluquinol may produce significant changes in the antiangiogenic activity of those derivatives, rendering either more active or less toxic multitargeted drug candidates for the treatment of angiogenesis dependent malignancies.

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

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Poster communication 28 – WG 2

Effects of Metformin on vascular reactivity in Streptozotocin-induced diabetic rats.

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Background : Diabetes is a metabolic disease that affects millions of people all through the world and is characterized by high glucose concentration. Many researches have shown that diabetes can lead to vascular dysfunction, DNA damage, oxidative stress and diabetic complications. Metformin is a commonly used drug that provides benefits on glucose metabolism and diabetes-related complications. The underlying mechanisms of these benefits are complex and still not understood completely. The aim of this research is to elucidate the role of complex mechanisms of metformin on vascular responses, which have not been fully understood until now, in these pathological conditions.

Methods : In order to determine the changes in endothelial and smooth muscle-induced relaxation responses, cumulative acetylcholine (10^{-9}-10^{-4} M) and salbutamol (10^{-9}-10^{-5} M) responses were obtained in the presence of L-NAME which is NO inhibitor in rat aorta tissues. Male Wistar rats (N=24) were injected with streptozotocin (STZ) (60 mg/kg, i.p.) or vehicle and the rats were divided into group 1 (control, n=6 group 2 (diabetes, n=6), group 3 (metformin, 100 mg/kg/day oral gavage, n=6), and group 4 (diabetes plus metformin 100 mg/kg/day oral gavage, n=7). The diabetic and healthy control rats were sacrificed on day 14 following STZ induction.

Results : Significant decrease in acetylcholine and salbutamol relaxation was observed in the diabetes group compared to the control group. Metformin restored endothelium-dependent relaxation response significantly. In addition, metformin restored the smooth muscle-dependent relaxation via β_2 adrenergic receptors. Phenylephrine- and serotonin-induced contraction responses tend to impair in diabetic group compared to control group, but results were not significant. Our results suggest that metformin can be beneficial on vascular reactivity responses which are impaired in diabetes.

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Poster communication 29 – WG 2

Hydrogen sulfide-targeted molecules may be new therapeutic targets for pulmonary arterial hypertension

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Background and Objectives: Pulmonary arterial hypertension (PAH) is a multifactorial disease resulting in progressive right heart failure characterized by increased pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR)[1]. In recent years, PAH treatment has progressed considerably with the development of targeted treatment protocols, but it is still a disease associated with high mortality and morbidity today[2]. The aim of our study was to examine the role of H_2S in the model of MCT-induced PAH in rats through both hypertension and Ach-mediated responses. .

Methods: Male Wistar rats (250-300 g) were divided into four groups: control, MCT, MCT+Na_2S and Na_2S. MCT (60 mg/kg, i.p.) was administered to rats as a single dose for induces the experimental PAH model. Physiological saline solution to control and MCT groups; Na_2S (2.5 mg/kg) solution to MCT+Na_2S and Na_2S groups were administered i.p. for 21 days. Under anaesthesia, a 23 gauge needle placed into the right ventricle (RV) and right ventricle pressure (RVP) was measured. The ratio of right ventricle/(left ventricle+septum) (RV/LV+S) was calculated to determine the index of RV hypertrophy. The main PAs were used in vascular experiments. First, PAs were pre-contracted with KCl and relaxed with a single dose of ACh (10^{-5} M). To investigate endothelial function, cumulative ACh relaxations were taken in the presence of Phe (10^{-6} M). Dose response curve were obtained by using DMT ring myograph..

Results: RVP and RV/(LV+S) ratio was significantly increased in the MCT group compared to the control group while this pressure and ratio was significantly decreased in the MCT+Na2S group (P<0.01, One-way ANOVA). KCl contraction were reduced significantly in MCT group compared to control group. This decrease was normalized in MCT+Na2S group (P<0.001, One-way ANOVA). ACh relaxations after KCl and Phe pre-contractions were reduced in MCT group compared to the control group but improved in MCT+Na2S group (P<0.01, One-way ANOVA).

Conclusion: Our results suggest that Ach-mediated responses are impaired in PAH. *In vivo* Na_2S therapy, as a donor of H_2S, may ameliorate vascular dysfunction and pulmonary hypertension by increasing H_2S in the model of MCT-induced PAH. This shows the importance of H_2S-targeted therapies in PAH

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Poster communication 30 – WG 2

Effect of CORM-2 Molecule on Vascular Tonus in Penile Tissue.

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Background : Although it is a multidimensional disease developed by endothelial disorder on the basis of erectic dysfunction, it is considered as an early symptom of cardiovascular diseases such as hypertension and diabetes. The interaction between NO, H_2S and CO, known as gasotransmitters, has been the subject of many studies. The aim of this study was to investigate the effects of CO donor CORM-2 on relaxation responses in corpus cavernosum and its relationship with other gasotransmitters, NO and H_2S mediated responses.

Methods : In order to investigate the relationship between CO and H_2S and NO, cumulative CORM-2 responses were obtained in the presence of AOAA is H_2S inhibitor in mouse corpus cavernosum tissues. Cumulative CORM-2 responses to phenylephrine in tissues incubated for 30 minutes with 10 mM AOAA versus control groups in the first experimental groups It was obtained. In the second experimental protocol, cumulative CORM-2 responses were obtained in the presence of 10-4 M L-NAME (10-6 - 3.10-4 M) versus control groups.

Results : In the presence of H_2S inhibitor AOAA, CORM-2 responses differ in endothelial and non-endothelial tissues. In the presence of L-NAME, the CORM-2 responses significantly decreased the E_{max} values compared to the control group.

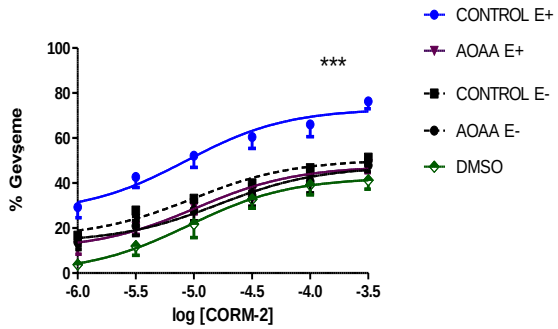

Figure 1: % Relaxation responses to cumulative CORM-2 after single dose 10-2 M AOAA incubation in mouse corpus cavernosum. p = 0.0001. Two-way ANOVA test. Bonferoni post test.

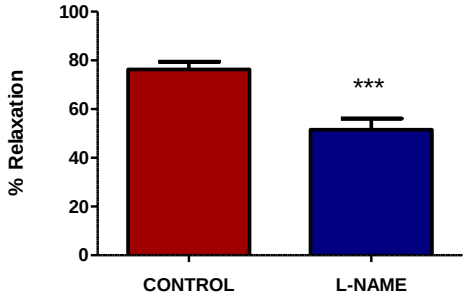

Figure 2: Emax % relaxation responses in the presence of L-NAME in the mouse coprus cavernosum. Data are given as mean ± standard error of mean. p = 0.0008 Control (n = 8) and L-NAME (n = 5) Unpaired Student's t Test.

Acknowledgement: This work was supported by a grant from Ege University Research Found (18ECZ001 to GOS)

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Poster communication 31 – WG 2

An old antibiotic have a potential for penile cancer treatment

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Heat-shock protein 90 (Hsp90) is a multi-functional chaperon protein with important roles in protein folding and keeping the stability of its client proteins. 17-Allylamino-17-Demethoxygeldanamycin (17AAG) is an antibiotic. 17AAG also binds to the ATP pocket on the N-terminus of Hsp90 and competitively inhibits ATP binding, which is essential to keep the client proteins of hsp90 in stability. We and others have shown that eNOS interacts with Hsp90 [1,2]. 17AAG has shown anti-tumorigenic effect via inhibition of eNOS-mediated angiogenesis pathway in myeloma [3,4]. However it is not studied well whether 17AAG also inhibits penile NOS. It is important since inhibition of eNOS or penile NOS have anti-tumorigenic effect by inhibition of angiogenesis in penis, which is important in penile cancer[6]. Squamous cell carcinoma of penile tissue is a rare disease usually treated with surgical intervention and treatment with chemotherapy was reported recently [5].

In our study, we first developed a voltammetric electrochemical biosensor detecting the activity of eNOS in homogenates of vascular tissues, including penile tissue. Isolated penile tissue strips were used for the study. Strips were incubated with either Krebs buffer with or without 17AAG (400nM, 15h). Strips were then homogenized. L-arginine (L-arg) and L-citrulline (L-cit) signals of homogenates were evaluated before and after incubation of homogenates with Ca^{+2}, L-arginine, NAPDH. In control homogenates, L-arg signal lowered and L-citrulline signal increased, whereas in 17-AAG treated homogenates, L-arg signals lowered again, but L-cit signals did not increased, compared to control group. This shows the inhibition of eNOS activity by 17AAG. L-arginine lowering in treated group might be associated with arginase activity, since arginase inhibitor treatment reversed the lowering of L-arginine signal (data not shown). This finding nominates 17AAG as a multi-target ligand for chemotherapy of penile cancer.

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Poster communication 32 – WG 3

In vitro effects of Eicosapentaenoic and Docosahexaenoic acid on the vascular tone of a human saphenous vein

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Introduction: Cardiovascular effects of omega-3 polyunsaturated fatty acids (PUFAs) including Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) have been widely reported[1-4]. However, little is known about the effect of EPA and DHA on human vascular tone [5-6]. Therefore, the aim of this study is to investigate the new biological target of EPA and DHA. Hereby, we aimed to evaluate the direct *in vitro* vascular effects of EPA and DHA on the human saphenous vein (SV) precontracted with either prostaglandin F2α (PGF2α), thromboxane A2 analogue (U46619) or norepinephrine (NE).

Methods: SV tissues were obtained from patients undergoing coronary artery bypass graft operation in the Department of Cardiovascular Surgery at Biruni University Hospital, Istanbul, Turkey. The Institutional Review Board of Haseki Research and Training Hospital, Istanbul, approved the study plan (Protocol no: 379). After removal of the surrounding connective tissue, SV was cut into rings of 3-5 mm width. Pretreatment of human SV rings with EPA and DHA (100 µM, 30 min) were tested on vascular reactivity induced by PGF2α (10 nM-5 µM), NE (10 nM-100 µM) and U46619 (1 nM-100 nM). In addition, direct relaxant effects of EPA/DHA (1-100 µM) were tested in human SV rings precontracted by PGF2α, NE, U46619. Furthermore, the involvement of potassium channels in their vascular effect was investigated in the presence of the non-selective K+ channel inhibitor tetraethylammonium chloride (TEA).

Results: EPA and DHA induced vasorelaxations in human SV precontracted with NE, $PGF_{2\alpha}$, and U46619. The relaxations were more prominent in $PGF_{2\alpha}$, and U46619 precontracted preparations than that of NE. In addition, pretreatment with these omega3-PUFAs decrease the contractile responsiveness of SV to NE, $PGF_{2\alpha}$, and U46619. The inhibitory effects of EPA and DHA were more pronounced on vascular tone mediated by prostanoid-receptors than that evoked by α-receptors in SV. Moreover, the direct vasorelaxant effect of EPA and DHA was abolished after use of a non-selective potassium channel inhibitor, namely TEA, particularly in U46619 precontracted preparations.

Conclusion: Our study demonstrates that both EPA and DHA reduce the increased vascular tone elicited by contractile agents on the human SV and that the direct vasorelaxant effect is likelyto involve potassium channels.

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Poster communication 33 – WG 4

A Phage Display derived-peptide targets specifically the VH1-69 BCR rearrangement in Chronic Lymphocytic Leukemia

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It is well demonstrated that the Chronic Lymphocytic Leukemia (CLL) is not only a monoclonal disease such as Multiple Myeloma (MM) but in the same patients could co-exist two or more neoplastic B cell clones. The unique biomarker which discriminates the single B cell sub-populations is the surface immunoglobulin B cell receptor (*sIgBCR*). The Ig-BCR includes the binding site (idiotype) for the epitope of cognate antigen, which results from stochastic and productive Ig variable genes rearrangement, and possible somatic hypermutations [1]. This genetic rearrangement of BCR during B cell maturation in CLL is not random as well as in physiological circumstances but leads to the assembly of so-called "stereotyped BCRs". In these last years, our researcher fields was based on different approaches both *in-vitro* and *in-silico*: by the phage display screening, we previously demonstrated the potential of small peptides to specifically bind the single IgBCRs as well as the selection of peptide binders of the idiotypic region of IgBCRs, called Id-peptides, while, by using the pharmacophore approach and the LB-3D-QSAR methodology, we investigated the common chemical features of Id-peptides sharing epitopic profile of CLL sub-populations and we designed new cyclic peptides structures useful for functional assays on primary CLL cells [2] [3]. In this work, we identify a 9-amino acids cyclic peptide able to target the un-mutated VH1-69 rearrangement in oligoclonal CLL patients, with no- interference with the other B cell sub-populations. Since the VH1-69 is often associated with a poor prognosis and fatal outcome, this targeting approach opens new strategies for a deeper single-B cell population analysis, especially the drug-resistance clones, during the course of the disease.

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Poster communication 34 – WG 4

3D-QSAR and design of new xanthene derivatives with enhanced antiproliferative activity

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Xanthene derivatives have become compounds of great importance in discovering of new anticancer drugs. Recent studies performed on 9-aryl substituted 2,6,7-trihydroxyxanthen-3-one and 3,3,6,6,-tetramethyl-9-aryl-substituted-xanthene-1,8(2H)-dione derivatives have shown their antiproliferative activity on HeLa cervical cell lines. Obtained IC_{50} values together with calculated molecular descriptors were subjected to Quantitative Structure Activity Relationship (QSAR) study in order to identify the most relevant molecular features responsible for the observed antiproliferative activity of compounds.

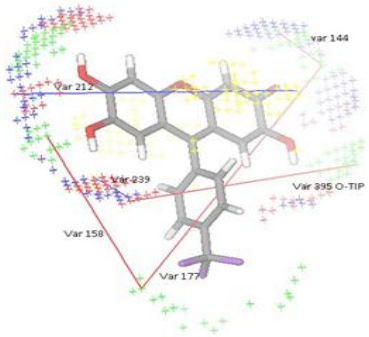

Figure 1: The most important molecular determinants responsible for antiproliferative activity.

The obtained QSAR model has shown next results: R^2=0.83, Q^2=0.951, RMSEE=0.147, R^2_{pred}=0.769, and RMSEP=0.207.

Based on the performed QSAR analysis and calculated ADMET properties, novel xanthene derivatives with enhanced antiproliferative activity were designed.

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