MEDCHEM2024

OHD ONE HEALTH DRUGS

XIII Meeting
Paul Ehrlich Euro-PhD Network & COST action OneHealth*drugs*

One Health approach to tackle neglected infectious diseases challenges

June 17-19, 2024

Rome, Sapienza University of Rome
Department of Chemistry & Technology for Drugs

Welcome message

The Organizing Committee cordially invites you to participate at **MedChem 2024** (XIII Meeting of the Paul Ehrlich Euro-PhD Network) to be held in Rome from the 17th to the 19th of June 2024 in conjunction with COST Action Workshop **One Health approach to tackle neglected infectious diseases challenges**.

As part of the COST Action workshop Young Researchers and Innovators will be engaged in the presentation of their research work in the field of neglected infectious diseases: Trypanosomiasis, Leishmaniosis, Schistosomiasis, Malaria and others in the field of medicinal chemistry/green chemistry and ecotoxicology. Designing lower environmental impact drugs.

The **MedChem2024** will be hosted by the Department of Chemistry and Technologies of Drugs, Sapienza University of Rome

The meeting will start on June 17th with the Opening Ceremony at the Aula Ginestra in the Stanislao Cannizzaro Building (CU014) located in the Sapienza Campus and will continue until 19th June.

The scientific contributions will cover all fields Medicinal chemistry. One section will be dedicated to the *COST Action* OneHealth*drugs Workshop*: "**One Health approach to tackle neglected infectious diseases challenges.**" (One Health Drugs).

The scientific contributions will be exhibited over three days, with the participation of distinguished international scientists and will host

- 5 plenary lectures by invited international key opinion leaders
- 31 oral communications
- 34 Flash poster communication
- Three poster sessions
- Paul Ehrlich MedChem Euro-PhD Network award exhibitions

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Stefano Alcaro
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Scientific programme

Day 1: Monday, June 17th

2.00 - 3.00 p.m CU019 building	Registration
3.00 - 3.30 p.m La ginestra (CU014)	Opening and welcome
	Chair: Elias Maccioni
3.30 - 4.00 p.m La ginestra (CU014)	Plenary lecture **Antivirals testing versus neurotropic viruses in single cell culture and in in vitro models of the blood brain barrier** **Joachim J. Bugert**, Bundewehr Institute of Microbiology, Munich, Germany
4.00 - 4.30 p.m La ginestra (CU014)	Invited lecture **Heterocycles Synthesis under Green Processes - Organometallic or Visible-light Catalysis.** **Philippe Belmont**, University Paris Cité, Paris, France
4.30 - 5.00 p.m La ginestra (CU014)	Flash Poster Presentation (FP1-FP12)
5.00 - 5.30 p.m	Coffee break and Poster session
5.30 - 7.00 p.m La ginestra (CU014)	Oral Communication (OC1-OC6)
OC-1	**B. Aguiar** Optimization and Characterization of Edaravone loaded Nanoparticles for the Treatment of ALS
OC-2	**B. Riss Yaw** Promising Antagonist for Neuropilin Receptor Targeting
OC-3	**F. Buonsenso** Recent Advances in Stimuli Responsive Iminosugars as Pharmacological Chaperones for Gaucher and Parkinson's Disease Treatment
OC-4	**G. Cernicchi** Potent 2-Phenyl-7-chloroquinazoline as new Mycobacterium avium Efflux Pump Inhibitors
OC-5	**A. Coco** Novel σ1R agonists/HDAC inhibitor codrugs modulating Tau protein
OC-6	**M. Coluccia** Design, synthesis and human monoamine oxidase inhibitory activity of 2 aroylbenzofuran derivatives: a new route towards hMAOs inhibition
7.00 - 9.00 p.m CU019 building	Welcome Party

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Day 2: Tuesday, June 18th, Morning session

	Chair: Serge van Calenbergh
9.00 - 9.30 a.m La ginestra (CU014)	Plenary lecture **Nucleotides as Antivirals - Development of membrane-permeable pronucleotides** **Chris Meier,** Universität Hamburg, Germany
	Chair: Maria Paola Costi - Daniela Secci
9.30 - 10.00 a.m La ginestra (CU014)	Invited lecture **Rohini R. Roopnarine**, School of Vet. Med, St. George's University, Grenada **COST Action Workshop "One Health approach to tackle neglected infectious diseases challenges"**
10.00-10.30 a.m La ginestra (CU014)	**E. Uliassi** introduction to COST YRI (young investigator and innovators) Flash poster presentation *Cost Action* (FP13-FP26)
10.30-11.00 a.m	Coffee break and Poster session
11,00-11,10 a.m La ginestra (CU014)	**S. Alcaro** The Chemotheca networking tool for the OneHealthDrugs *Cost Action*
	Chair: Stefano Alcaro
11.10 -1.10 p.m La ginestra (CU014)	Oral communication (OC7-OC14)
OC-7	**T. Quennesson** Design, synthesis and evaluation of reverse β-aza fosmidomycin analogues as 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR) inhibitors as antimicrobials
OC-8	**L. Tisseur** Modulating the 2-position of imidazo[1,2-a]pyrazine lead CTN1122 alters its antileishmanial properties and L-CK1.2 kinase inhibition profile
OC-9	**E. Sanna** Discovering potential Pan-hCoV inhibitors by targeting Nsp13 Helicase
OC-10	**M. Bufano** Novel FtsZ inhibitors identified by virtual screening and Adaptive Steered Molecular Dynamics
OC-11	**L. Márquez Cantudo** Insight into the catalytic mechanism of M17 leucine aminopeptidase through QM/MM computer simulations and selective mutagenesis
OC-12	**B. Nunes** Tailoring Quaternary Phosphonium Salts: Design, Synthesis, Antimicrobial and Cytotoxicity
OC-13	**M. Pacetti** Investigation of the Cycloheptathiophene-3-carboxyamide Core for the Discovery of Influenza Virus Polymerase inhibitors
OC-14	**E. Van de Velde** Synthesis and phenotypical discovery of imidazo[2,1-f]triazine ribosides as broad spectrum antitrypanosomal agents
1.10 - 2.15 p.m	Lunch

Day 2: Tuesday, June 18th, Afternoon session

	Chair: Athina Geronikaki
2.15 - 3.15 p.m Room A (CU019)	**Meeting of PE Coordinators/ Free time**
3.15 - 5.15 p.m La ginestra (CU014)	**Oral Communication** (OC15-OC22)
OC-15	**F. Falbo** Synthesis of new benzanilide derivatives as HDAC1 selective inhibitors for CRC treatment
OC-16	**A. Fanizzi** Rational optimization of the N-adamantyl-anthranil amide structural core for the developtment of new selective ligands for the Cannabinoid subtype 2 Receptor (CB2R)
OC-17	**A. Fontana** Semisynthetic usnic acid-based compounds as promising antifungal agents
OC-18	**R. Purgatorio** Investigating the multi-target potential for complex diseases of the alkaloidcontaining pyrrolo[2,1-a]isoquinoline scaffold
OC-19	**P. Sciò** Benchmarking a Deep Generative Model for the Optimization of 3-Aroyl-1,4-diarylpyrroles (ARDAP)
OC-20	**A.R. Gomes** Discovery of a potent steroidal oxime against breast and lung cancers
OC-21	**R. Astolfi** Quantitative Composition-Activity Relationships Through Machine Learning Algorithm. Application to Essential Oil Tested as Acetylcholinesterase Inhibitors
OC-22	**L. Lombardo** Designing an Effective Pharmacophore Model for Sigma 1 Receptor
5.15 - 5.30 p.m La ginestra (CU014)	**Flash Poster communication** (FP27-FP34)
5.30 - 6.00 p.m	**Coffee break and Poster session**
	Chair: Romano Silvestri
6.00 - 7.15 p.m La ginestra (CU014)	**Oral communication** (OC23-OC27)
OC-23	**E. Marchese** Revealing Polyphenols' Therapeutic Potential in Multiple Myeloma: Insights from Computational Studies on 20S Proteasome
OC-24	**G. Buttitta** Scalable microfluidic method for tunable liposomal production by a rational approach
OC-25	**I. Sardo** Natural F-series Pyrrolomycins: Microwave-assisted total synthesis, anticancer activity, and mechanism of action
OC-26	**C. Sarnari** Progresses in targeting mono-ADP ribosylating enzymes: towards selective inhibitors
OC-27	**B. Serambeque** Photodynamic therapy for endometrial cancer: can aldehyde dehydrogenase play a key role in eliminating cancer stem cells?
8.30 p.m	**Social Dinner at Casale di Tor di Quinto**

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Day 3: Wednesday, June 19th

	Chair: Fernanda Borges
9.30 - 10.00 a.m La ginestra (CU014)	Plenary lecture **Kick-start new drug design projects using computer-aided drug design** **Andrea Brancale**, University of Chemistry and Technology Prague, Czech Republic
10.00-10.45 a.m La ginestra (CU014)	Oral communication (OC28-OC31)
OC-28	**M. Garbagnoli** Discovery of SMol acting on HuR-RNA complexes. STD-NMR competition studies using Peptide Nucleic Acids
OC-29	**A. Gargano** Drug discovery of natural agents endowed with anticancer properties
OC-30	**R. Rocca** Targeting TERRA G-quadruplex: Design and Biophysical Evaluation of Chromene Scaffold Derivatives
OC-31	**E. L. Citriniti** Lithospermic Acid: Unveiling Its Potential as a Pancreatic Lipase and hCA V Inhibitor through In Silico and In Vitro Studies
10.45- 1.15 a.m	Coffee break
11.15-12.45 a.m La ginestra (CU014)	PE MedChem Euro-PhD Label communications PEA1-PEA6
PEA-1	**C. Gratteri** Development of multi-target agents with anti-cancer activity
PEA-2	**A. Olejarz-Maciej** Multidirectional activity of new histamine and adenosine receptor ligands
PEA-3	**A. Onali** Application of computational and synthetic techniques for the identification of compounds with potential antitumour and antiviral activity
PEA-4	**V. Pontecorvi** From Hypoxia to Human Carbonic Anhydrases: design, synthesis, andevaluation of novel pyran-2-one basedderivatives as anti-tumour and anti-inflammatory agents
PEA-5	**F. Procopio** Molecular Modelling for medicinal chemistry: Development and application
PEA-6	**P. Stępnicki** Optimization of virtual hits D2AAK3 and D2AAK2 in the search for new drugs for the treatment of schizophrenia
12.45 - 1.15 p.m	Closing remarks

Oral communication

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OC1

Optimization and Characterization of Edaravone loaded Nanoparticles for the Treatment of ALS

Brandon Aguiar,[a,b,c] Rita Alfenim,[a] Cláudia Machado,[a] Renata Silva,[b,c] Fernando Remião,[b,c] Francisco Otero-Espinar,[d] Fernanda Borges,[a] Carlos Fernandes[a]

[a] CIQUP-IMS – Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, R. Campo Alegre s/n, 4169-007 Porto, Portugal.
[b] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.
[c] UCIBIO – Applied Molecular Biosciences Unit, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.
[d] Pharmacology, Pharmacy and Pharmaceutical Technology Department, Faculty of Pharmacy, University of Santiago de Compostela (USC), 15782 Santiago de Compostela, Spain.

brandonlageaguiar@gmail.com

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive neurodegenerative disorder leading to death within 2 to 5 years after symptom onset (*1*). At the present moment, treatment options such as Edaravone (EDV), are limited due to incapacity to effectively cross the Blood-Brain-Barrier, short-life span and poor stability. The scope of this work was to encapsulate EDV in different drug delivery systems (DDS) based on polymeric (PNP), lipid-polymer hybrid (HNP) nanoparticles and nanostructured lipid carriers (NLC). For that, an extensive optimization was performed by varying synthetic parameters such as DDS's backbone, the presence or absence of PEG chains in DDS surface, the polymer or lipid/drug ratio and the pH of aqueous phase, being the PNP and HNP synthetized by the nanoprecipitation method and the NLC prepared by the solvent emulsification/evaporation method. EDV was successfully encapsulated in all tested DDS, being their size, polydispersity and surface charge analyzed using a zetasizer analyzer. It was also quantified the amount of EDV entrapped in DDS and the biocompatibility of the resulting nanoformulations was evaluated using a human neuroblastoma (SH-SY5Y) cell line. Overall, the use of PEG or TPGS led to a decrease in nanoparticle size, for the PNPs and HNPs, as well as a decrease in the negative surface charge. When high pH was used in aqueous phase in the synthesis of PNP, it was observed a decrease of nanoparticle size, followed by an instability of EDV. Overall, the DDS tested did not show cytotoxicity in the SH-SY5Y cell line with exception for NLCs formulations that showed some cytotoxicity possibly due to lipidic peroxidation. In conclusion, this work shows that EDV can be encapsulated in different nanocarriers that could act as an interesting alternative for the treatment of ALS.

Acknowledgments: This work was funded by FEDER funds through national funds by FCT – Foundation for Science and Technology under research grants UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS) and 2021.04016.CEECIND/CP1655/CT0004. A.R.A (2023.01250.BD), B.A. (2020.08731.BD), were supported by FCT. C.F. thanks the FCT for the financial support of his work contract through the Scientific Employment Stimulus—Individual Call (2021.04016.CEECIND/CP1655/CT0004).

1. G. Y. Wang, S. L. Rayner, R. Chung, B. Y. Shi, X. J. Liang, Advances in nanotechnology-based strategies for the treatments of amyotrophic lateral sclerosis. Mater Today Bio 6, 100055 (2020).

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OC2

Promising Antagonist for Neuropilin Receptor Targeting

RISS YAW Benjamin,[a] LAMAA Diana,[a] KATARZYNA PUSZKO Anna,[b] LEPELLETIER Yves,[c] DEMANGE Luc.[a]

[a] Université de Paris Cité, CiTCoM, UMR CNRS 8038, department of Faculté de Pharmacie
4 avenue de l'observatoire, 75006 Paris, France

[b] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

[c] Laboratory of Cellular and Molecular Mechanisms of Hematological Disorders and Therapeutical Implications, INSERM UMR1163/CNRS URL 8254, Paris, France

benjamin.riss-yaw@u-paris.fr

Neuropilins are co-receptors of VEGF-A165, which are responsible for angiogenesis, and are over-expressed on the surface of tumor cells. Targeting NRP is of major interest for the development of chemotherapeutic strategies capable of treating angiogenesis-related pathologies. We set out to identify a non-peptide antagonist of NRP/VEGF-A165 binding that would display anti-angiogenic and anti-tumor activities *in vivo*. To determine the structure-activity relationship of two models identified, we synthesized a scale-up of molecules. We were able to demonstrate cytotoxic effects on HUVEC and MDA-MB-31 cells, and antagonize NRP/VEGF-A165 binding through biological evaluation of these structures. This study allowed us to identify the key structure of two antagonists, NRPa-47 and NRPa-308,[1-2] that are selective for NRP/VEGF-A165, paving the way for the synthesis of promising antitumor drugs based on targeting the NRP/VEGF-A165 association.

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[1] Liu, W. -Q.; Megale, V.; Borriello, L.; Leforban, B.; M. Montès; Goldwaser, E.; Gresh, N.; Piquemal, J. -P.; Hadj-Slimane, R.; Hermine, O.; Garbay, C.; Raynaud, F.; Lepelletier, Y.; Demange L. *Cancer Lett.* **2014**, *349* (*2*), 120-127.

[2] Liu, W. -Q.; Lepelletier, Y.; M. Montès; Borriello, L.; Jarray, R.; Grepin, R.; Leforban, B.; Loukaci, A.; Benhida, R. ; Hermine, O.; Dufour, S.; Pages, G.; Garbay, C.; Raynaud, F.; Hadj-Slimane, R.; Demange L. *Cancer Lett.* **2018**, *414*, 88-98.

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OC3

Recent Advances in Stimuli Responsive Iminosugars as Pharmacological Chaperones for Gaucher and Parkinson's Disease Treatment

Fabio Buonsenso,[a] Francesca Clemente,[a] Francesca Cardona,[a] Martina Cacciarini,[a] Andrea Goti,[a] Camilla Matassini[a]

[a] Department of Chemistry 'Ugo Schiff', University of Florence

via della Lastruccia 3-13, 50019 Sesto Fiorentino (FI), Italy

fabio.buonsenso@unifi.it

Gaucher disease (GD), the most common lysosomal storage disorder, is caused by mutations in the *GBA* gene, leading to misfolding of the enzyme acid-β-glucosidase (GCase) and resulting in substrate accumulation and severe multisystemic symptoms. More recently, GCase has been shown to be involved in the pathogenesis of Parkinson's disease, thus linking the two diseases through common molecular mechanisms and suggesting a potential shared cure.[1] A promising therapeutic option is based on Pharmacological Chaperones (PCs), small compounds able to bind GCase in the endoplasmic reticulum (pH = 7) and assist its trafficking to the lysosome (pH = 4.5), where they are replaced by the natural substrate, thus rescuing enzyme activity (Figure 1).[2] To maximise the chaperoning activity, this study aims to develop innovative PCs based on smart host-guest systems that respond to physiological pH fluctuations. We synthesized the guest compounds, namely iminosugars with piperidine and pyrrolidine skeleton derived from cost-effective carbohydrates, such as D-mannose, D-glucose and D-arabinose. These compounds were proven to enhance GCase activity in cell lines from Gaucher patients and will use in complexation experiments with appropriate host systems.

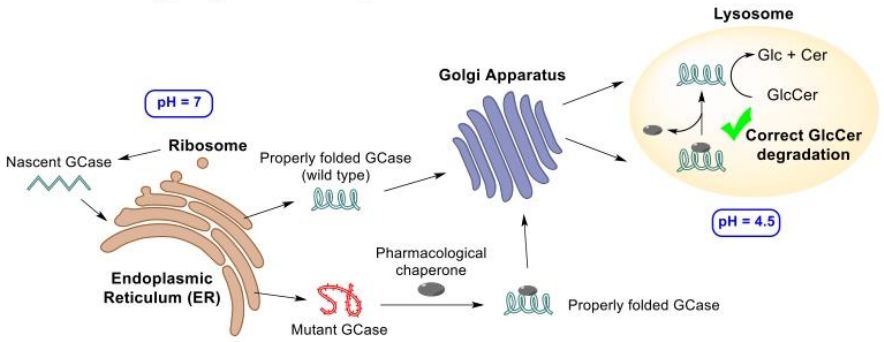

Figure 1. Representative illustration of the mode of action for PCs[2]

Acknowledgement: Fondo per il Programma Nazionale della Ricerca (PNR) e Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN), finanziato dall'Unione europea – NextGenerationEU. Project: PH-PRISM, CUP: B53D23015340006. Si ringrazia il Fondo di Beneficenza ed opere di carattere sociale e culturale di Intesa Sanpaolo (erogazione B2021/0187).

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[1] S. R. L. Vieira, A. H. V. Schapira, *J. of Neural Transm.*, **2022**, *129*, 1105.

[2] M. Martínez-Bailén, F. Clemente, C. Matassini, F. Cardona, *Pharmaceuticals*, **2022**, 15, 823.

OC4

Potent 2-Phenyl-7-chloroquinazoline as new *Mycobacterium avium* Efflux Pump Inhibitors

Giada Cernicchi,[a] Tommaso Felicetti,[a] Violetta Cecchetti,[a] Elisa Rampacci,[b] Laura Rindi,[c] Stefano Sabatini[a]

[a] Dept. of Pharmaceutical Sciences, Università di Perugia, Via del Liceo 1, 06123, Perugia, Italy

[b]Dept. of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy

[c]Dept. of Translational Research and New Technologies in Medicine and Surgery, Università di Pisa, Via San Zeno, 35/39, I-56127, Pisa, Italy

giada.cernicchi@outlook.it

Belonging to Nontuberculous mycobacteria (NTM), *Mycobacterium avium* complex is classified as a difficult-to-treat pathogen due to its ability to rapidly develop drug resistance to clarithromycin (CLA), the most used antibiotic to treat NTM infections. Overexpression of efflux pumps (EPs) has been shown to be a key mechanism of CLA resistance in NTM.[1] Due to the lack of in-depth knowledge of the structure of NTM EPs, only a few molecules characterized by a narrow chemical diversity have been reported as NTM EPIs.[2-4] Therefore, in this work, a set of compounds from an *in-house* library, was selected based on chemical diversity to be tested as potential NTM EP inhibitors (EPIs) against *M. smegmatis* mc^{2}155 and *M. avium* clinical isolates. Considering the obtained results, analogues of the best derivatives **1b** and **7b** were designed and synthesized leading to compound **13b** which emerged as the most potent NTM EPI able to reduce the CLA MIC by 16-fold when tested at 4 µg/mL against the clinical isolate *M. avium* 2373 which overexpresses EPs as the primary mechanism of CLA resistance.

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[1] L. Rodrigues *et al.*, *Int. J. Antimicrob. Agents*. **2009**, *34*, 529 – 533.

[2] L. Rindi, *Int. J. Mol. Sci*. **2020**, 21, 1 – 13.

[3] R. Cannalire *et al.*, *Eur. J. Med. Chem*. **2017**, 140, 321 – 330.

[4] T. Felicetti *et al.*, *ACS Infect. Dis*. **2019**, 5, 982 – 1000.

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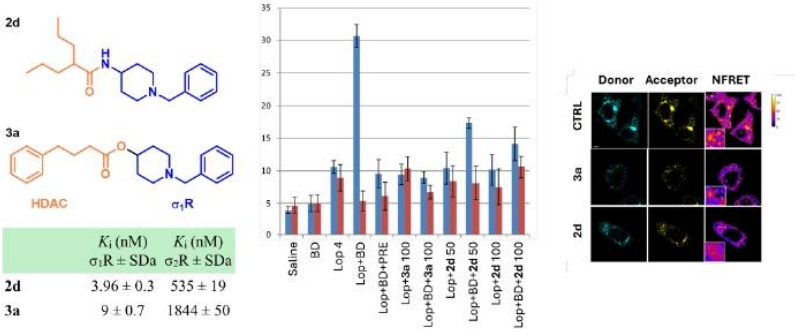
OC5

Novel σ_1R agonists/HDAC inhibitor codrugs modulating Tau protein

Alessandro Coco[a], Carla Barbaraci[a], Antonino Fallica[a], Arianna Scarlatti[b], Giacomo Siano[c], Giorgia Giordano[a], Emanuele Amata[a], Lorella Pasquinucci[a], Enrique José Cobos[d], Ivana Cacciatore[e], Antonio Di Stefano[e], Cristina Di Primio[c], Agostino Marrazzo[a]

[a] Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy; [b] Scuola Normale Superiore, Pisa 56124, Italy; [c] Institute of Neuroscience, Italian National Research Council (CNR), Pisa 56124, Italy; [d] Department of Pharmacology, School of Medicine, University of Granada, 18016 Granada, Spain; [e] Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Chieti Scalo, Italy.
alessandro.coco@phd.unict.it

Among the various neurodegenerative diseases, Alzheimer's Disease (AD) is the most common, representing millions of cases worldwide. AD is caused by the deposition of protein aggregates represented by Aβ amyloid plaques and neurofibrillary tangles, mainly tau protein. In this context, σ_1R agonists and σ_2R antagonists have been shown to exert neuroprotective effects preventing tau hyperphosphorylation.[1] Additionally, HDAC enzymatic activity can promote tau cytotoxicity and neurodegeneration in an isoform-dependent manner. Herein we propose novel σR/HDACi derivatives synthesized through esterification or amidation of valproic acid (VPA) and phenyl butyric (PHB) acid and σ moieties. Among the synthesized compounds **2d** and **3a** are overall the most interesting with K_i values in the nM range for σ_1R and high selectivity compared to σ_2R, as shown in the **figure**. Stability studies and ADMET prediction show that **2d** has greater stability, unlike **3a** which undergoes immediate hydrolysis, and both compounds cross the BBB. The latency test with loperamide and BD-1063 depicted a profile for compounds **2d** and **3a** aligned with σ_1R agonists. Finally, the assay to evaluate the ability of both compounds to prevent tau aggregation was performed using cells expressing the FRET CST P301S biosensor that behaves like the endogenous tau protein. Our analysis focused on the mean NFRET of intracellular aggregates, providing insights into their stability. Cells treated with compounds **2d** and **3a** show a reduction in NFRET indicating that aggregates are less stable compared to the control. In conclusion, compounds **2d** and **3a** will be used for future tests and validations.



[1] Lahmy, V.; Meunier, J.; Malmstrom, S.; Naert, G.; Givalois, L.; Kim, S. H.; Villard, V.; Vamvakides, A.; Maurice, T., Neuropsychopharmacology **2013**, 38 (9), 1706-1723.

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OC6

Design, synthesis and human monoamine oxidase inhibitory activity of 2 aroylbenzofuran derivatives: a new route towards hMAOs inhibition

Michele Coluccia[a], Paolo Guglielmi[a], Guya Diletta Marconi[b], Francesca Diomede[b], Francesca Arrighi[a], Daniel Chavarria[c], Arianna Granese[a], Fernanda Borges[c], Daniela Secci[a]

[a] Department of Pharmaceutical Chemistry and Technology, Sapienza University of Rome, P.le A.Moro 5, 00185 Rome, Italy

[b] Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy

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

michele.coluccia@uniroma1.it

Alzheimer's and Parkinson's diseases are characterized by neuronal loss and degeneration of behavioural and cognitive functions, increased difficulty in movement, deterioration of life quality of patients and an overall degeneration of the central nervous system. Monoamine neurotransmitters, such as dopamine and noradrenaline, play a pivotal role in neuromodulation and cognitive functions. Human monoamine oxidases (hMAOs) are the main enzymes involved in their metabolization and, especially the B isoform, seem to contribute to the pathogenesis of neurodegenerative conditions[1,2]. Because of this and based on the previous development of hMAO-B inhibitors with different scaffolds by our research group, we have designed, synthesized and characterized novel 2-aroylbenzofuran derivatives with anti-hMAO-B activity. The compounds have been synthesized using an easy one-step process. The biological activity of the compounds has then been assessed through the measurement of their IC_{50} and cellular assays. The compounds have shown an overall good yield and, among the developed compounds, two of the 2-aroylbenzofuranes have shown a promising IC_{50} in the nanomolar range. The most promising derivatives have been preliminary evaluated on human gingival fibroblasts (hGFS) and the neuroblastoma SH-SY5Y cell line, an established neuronal cell model employed to perform PD research. Most of the compounds were shown to be safe and not significantly altering the cell viability.

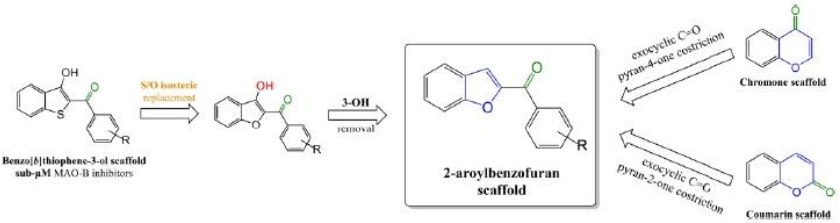

Fig. 1: Design scheme of the 2-aroylbenzofuran scaffold

[1] Aksoz BE, Aksoz E. *Comb Chem High Throughput Screen*. 2020;23(9):877-886

[2] Yi C, Liu X, Chen K, Liang H, Jin C.. *Eur J Med Chem*. 2023;252

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OC7

Design, synthesis and evaluation of reverse β-aza fosmidomycin analogues as 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR) inhibitors as antimicrobials

Thibaut Quennesson,[a] Martijn Risseeuw [a], Serge Van Calenbergh[a]

[a] Laboratory for Medicinal Chemistry, Ghent University

Ottergemsesteenweg 460, B-9000 Ghent, Belgium

Thibaut.quennesson@UGent.be

The rapid emergence and proliferation of multi-drug-resistant strains of pathogenic bacteria and parasites presents a global threat to human health. Antimicrobial resistance could potentially become the leading cause of deaths by 2050[1]. There is an urgent need for the development of new antimicrobial agents featuring innovative mechanisms of action. One promising target in this pursuit is the enzyme known as 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR), responsible for the second step in the methylerythritol phosphate (MEP) pathway, which is essential for numerous pathogens but absent in humans[2]. Fosmidomycin is a natural potent DXR inhibitor known to be a safe and effective antimalarial[3]. Nonetheless, its clinical potential is limited by suboptimal pharmacokinetic properties and poor bacterial uptake. Inspired by glyphosate, a well-known herbicide, and the eNTRy rules, we have rationally designed and synthesized a series of reverse β-aza fosmidomycin analogues with the objective of enhancing their permeability and improving their ADMET properties. A reliable, time-efficient and cost-efficient synthetic route was achieved through the Kabashnik-Fields reaction, yielding potent and selective DXR inhibitors.

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OC8

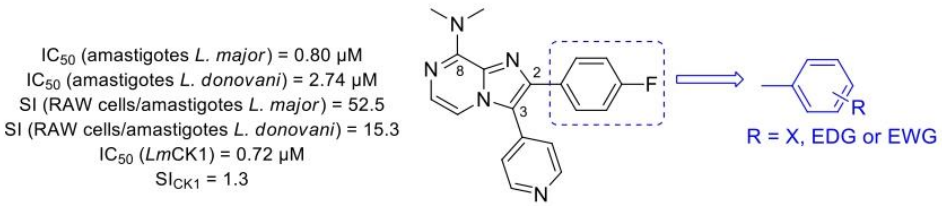
Modulating the 2-position of imidazo[1,2-*a*]pyrazine lead CTN1122 alters its antileishmanial properties and L-CK1.2 kinase inhibition profile

Lhana Tisseur,[a] Marc-Antoine Bazin,[a] Sandrine Cojean,[b] Fabrice Pagniez,[a] Guillaume Bernadat,[b] Cédric Logé,[a] Christian Cavé,[b] Carine Picot,[a] Olivier Leclercq,[c] Blandine Baratte,[d] Najma Rachidi,[c] Stéphane Bach,[d] Philippe Loiseau,[b] Patrice Le Pape,[a] Pascal Marchand[a]

[a]Nantes Université, Cibles et médicaments des infections et de l'immunité, IICiMed, UR 1155, F-44000 Nantes, France.
[b]Université Paris-Saclay, Chimiothérapie Antiparasitaire, BIOmolécules : Conception, Isolement et Synthèse – BioCIS UMR CNRS 8076, F-91400 Orsay, France.
[c]Institut Pasteur and INSERM U1201, Unité de Parasitologie Moléculaire et Signalisation, F-75015 Paris, France.
[d]Sorbonne Universités, UPMC Paris 06, CNRS USR 3151 "Protein Phosphorylation and Human Disease" group, Station Biologique, F-29680 Roscoff, France.

Correspondence: lhana.tisseur@etu.univ-nantes.fr

Leishmaniasis is a parasitic disease considered as a neglected tropical disease by the WHO[1]. It occurs in various forms - cutaneous, mucocutaneous and visceral - and constitutes a serious public health problem, with 12 millions of people infected in the world and more than 40,000 deaths per year. The disease is endemic in many parts of the world, and its emergence in Europe is attributed to global warming. Existing treatments are far from optimal, with high toxicity, high costs and administration that limits their use by disadvantaged populations. In addition, widespread parasite resistance is arising to these treatments, reducing their effectiveness in certain regions. There is therefore an urgent need to develop new, safer and more effective treatments, targeting new proteins to overcome these resistances. A promising recent discovery is the **CTN1122**[2], which targets a specific *Leishmania* Casein Kinase 1 protein (L-CK1.2)[3,4], and shows good antileishmanial properties. In this context, we decided to synthesize **CTN1122** analogues in order to improve the pharmacological activity profile. (**Figure 1**)



CTN1122

Figure 1: Modulation from the lead compound **CTN1122**

Via an original synthesis strategy, fifteen new analogues resulting from the optimization of **CTN1122**, by the modification of the substituent in positions 2 of the imidazo[1,2-*a*]pyrazine ring, were obtained. The study of these analogues will allow discussing the structure-activity relationship regarding their antileishmanial properties, their L-CK1.2 target protein inhibition capacities and taking into account their toxicity profile.

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OC9

Discovering potential Pan-hCoV inhibitors by targeting Nsp13 Helicase

Erica Sanna[a], Angela Corona[a], Alessia Onali[a], Rita Meleddu[a], Giulia Atzeni[a], Laura Demuru[a], Enzo Tramontano[a], Simona Distinto[a], Filippo Cottiglia[a], Antonio Lupia[a], Elias Maccioni[a]

[a] Department of Life and Environmental Sciences, University of Cagliari

A Building, Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari, Italy

erica.sanna@unica.it

Helicase of Human Coronaviruses (hCoVs) utilizes the energy of nucleotide triphosphate hydrolysis to catalyse the unwinding of double-stranded DNA or RNA in a 5' to 3' direction. Due to its highly conserved sequence and crucial role in viral replication, it is an attractive and promising target for drug development against various human coronaviruses (hCoVs), which may lead to mild self-limiting respiratory or serious infections and deadly diseases. [1]

In this work, with the aim of identifying new potential Pan-hCoV helicase inhibitors, crystallographic structures of SARS-CoV-2 helicase available to date [2, 3] have been considered to rationally design a library of about 100 compounds that have been subsequently synthesized and characterized by structural (single-crystal X-ray diffraction) and spectroscopic (NMR, MS) methods. All compounds were found to inhibit both SARS-CoV-2 helicase-associated enzyme activities, namely NTPase and unwinding activity, showing IC_{50} values in the low micromolar range; among them, several compounds inhibited SARS-CoV-2 replication with low EC_{50} and no significant CC_{50} values. In addition, some of the most potent compounds exhibited pronounced antiviral activity against HCoV229E and MERS-CoV, highlighting some of them as promising Pan-hCoV helicase inhibitors.

These results suggest that the hCoV helicase is a valid target for the development of new drugs to treat infection by SARS-CoV-2 and other HCoVs, potentially causing future emerging and re-emerging infectious coronavirus diseases.

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OC10

Novel FtsZ inhibitors identified by virtual screening and Adaptive Steered Molecular Dynamics

Marianna Bufano,[a] Pietro Sciò,[a] Silvia Buroni,[b] Antonio Coluccia[a]

[a] *Laboratory Affiliated with the Institute Pasteur Italy-Cenci Bolognetti Foundation, Department of Drug Chemistry and Technologies, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Roma, Italy*
[b] *Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Via Adolfo Ferrata 9, 27100, Pavia, Italy*

marianna.bufano@uniroma1.it

Filamentous temperature-sensitive Z (FtsZ) protein represents a promising antibacterial target owing to its essential role in bacterial cell division. Serving as the prokaryotic functional homologue of tubulin, FtsZ possesses the capability to polymerize into protofilaments orchestrating the formation of the Z-ring during binary fission [1]. A molecular docking and pharmacophore based virtual screening campaign resulted in the discovery of a novel FtsZ polymerization inhibitor (compound 11) exhibiting an IC_{50} potency value of 48 µM and an *in-vivo* MIC of 2 mg/mL for *S. aureus*. To enhance potency and diminish affinity towards efflux pumps, an Adaptive Steered Molecular Dynamics (ASMD) based drug design approach was employed.

ASMD [2] presents a refinement of the Steered Molecular Dynamics (SMD) technique where the driving of a steered particle (i.e. the unbinding of a ligand) is performed by a time-dependent harmonic force in stages. Unlike SMD, which necessitates an extensive number of replicas to encompass all potential unbinding configurations and generate a viable Potential of Mean Force (PMF) along the reaction coordinates using Jarzynski's Equality (JE), ASMD streamlines this process. This is accomplished by segmenting the unbinding pathway into stages and discarding trajectories that significantly deviate from the equilibrium path at the conclusion of each stage.

Structural analogs of compound 11 were purchased and tested to build preliminary structure-activity relationships (SAR). Utilizing the ASMD procedure, we were able to retrospectively predict the trend of inhibitory activities exhibited by these molecules. The optimized parameters derived from this analysis were then employed to design a new derivative featuring chemical moieties tailored towards mitigating bacterial efflux and enhancing potency.

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OC11

Insight into the catalytic mechanism of M17 leucine aminopeptidase through QM/MM computer simulations and selective mutagenesis

Laura Marquez-Cantudo[1], Claire Coderch[1], Alejandra Álvarez[2], Rubén Agudo[2], Carmen del Águila[2], Ana Ramos[1], Beatriz de Pascual-Teresa[1], Elizabeth Valdivieso[2]

[1] Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28668, Boadilla del Monte, Spain.
[2] Departamento de CC, Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28668 Boadilla del Monte, Spain.

laura.marquezcantudo@ceu.es

Acanthamoeba castellanii is an emerging parasite that causes fatal granulomatous encephalitis and amoebic keratitis in humans. During its life cycle, when the conditions are not favorable, *Acanthamoeba* transforms into a cyst that is able to protect the parasite from the immune response, disinfectants, and chemotherapeutic agents.[1] During the encystation process, the expression levels of M17 leucine aminopeptidase (M17LAP) are increased and its knockdown has been related to an impairment of the cyst maturation.[1] M17LAP is a metalloexopeptidase that removes the N-terminal amino acid of peptides (usually a leucine) by a catalytic mechanism involving two adjacent zinc ions (Zn^{2+}) in its active site at an optimum pH of 8.5.[2] The substrate-like ligand Leu-AMC (leucyl aminocoumarin) is commonly used to measure the catalytic activity of M17LAP in *in vitro* assays. The consensus for the catalytic mechanism is that a hydroxide anion issued from a water molecule attacks the carbonyl carbon of the scissile amide bond rendered highly electrophilic by the coordination to Zn^{2+}. Nevertheless, there is discrepancy on the formation of such hydroxide anion. Several proposals involve a water molecule from the coordination sphere, together with the intervention of either a lysine from the active site (K262 in bovine, K357 in *Acanthamoeba*) or a bicarbonate ion.[3,4] Here we describe the generation of an AlphaFold model of M17LAP followed by hybrid QM/MM simulations (SCC-DFTB3/AMBER). Based on the analysis of these simulations, we propose that the side chain of K357 acts as a general base for the deprotonation of a solvent water molecule. This reaction mechanism could be common to all M17LAP whether they have water molecules in the coordination sphere or not, as this proposed mechanism relies only on the water molecules from the surrounding solvent. These results have been further corroborated by experiments involving selective mutations of the above mentioned K357. The proposed catalytic mechanism for M17LAP has allowed us to guide the rational design of inhibitors for this target, which has already yielded promising hit compounds.

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OC12

Tailoring Quaternary Phosphonium Salts: Design, Synthesis, Antimicrobial and Cytotoxicity

Bárbara Nunes [a,b,c], Fernando Cagide [c], Carlos Fernandes [c], Fernanda Borges [c], Manuel Simões [a,b]
[a] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[b] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[c] CIQUP-IMS, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal
up201804372@edu.fe.up.pt

Antimicrobial resistance (AMR) poses a major global health threat, as evidenced by the impact of ESKAPE pathogens, which limit treatment options, increase disease burden, and elevate mortality rates. Consequently, there is an urgent need for new antimicrobial agents, with quaternary ammonium (QAS) and phosphonium (QPS) compounds emerging as promising candidates [1,2]. Our research aims to identify molecular features of QAS/QPS compounds for the rational design of new derivatives with improved antimicrobial action. Previous studies [1,2] have shown the efficacy of dodecyl and tetradecyl triphenylphosphonium derivatives (H-TPP-DC and H-TPP-TD) against *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, and *Candida albicans*, but have also noted significant cytotoxic and hemolytic effects. To mitigate toxicity, we envisioned the substitution of the TPP^+ phenyl rings with electron-withdrawing and electron-donating groups. Herein, we present (i) the synthesis of these substituted TPP^+-dodecyl and -tetradecyl compounds, (ii) their antibacterial activity against antibiotic-susceptible and resistant *S. aureus* strains, and (iii) their *in vitro* cytotoxicity assessment. The following substituents were chosen for the experimental study: $-CF_3$ (strong electron-withdrawing inductive effect), –F (moderate electron-withdrawing inductive effect), and $-OCH_3$ (strong electron-donating resonance effect). To minimize steric hindrance and obtain the maximum charge (de)stabilizing effects, only *para*-substituted TPP^+ derivatives were synthesized. The substituted TPP^+ salts exhibited relevant antibacterial activities against *S. aureus* comparable to the salts with a TPP^+ cation (H-TPP-DC and H-TPP-TD). Cell viability assays on human hepatocarcinoma cells revealed that derivatives with electron-withdrawing substituents, 4-CF_3-TPP-DC and 4-CF_3-TPP-TD, had reduced toxicity and lower intracellular reactive oxygen species production compared to H-TPP-DC and H-TPP-TD. Thus, the 4-CF_3-TPP^+ moiety emerged as a superior alternative to TPP^+ for developing new antimicrobial agents.

Acknowledgments: This work 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 EXPL/MED-QUI/1156/2021, UIDB/00081/2020 (CIQUP), PTDC/ASPPES/28397/2017, LA/P/0056/2020 (IMS), LA/P/0045/2020 (ALiCE), UIDB/00511/2020, UIDP/00511/2020 (LEPABE), PTDC/BII-BTI/ 30219/2017 - POCI-01-0145-FEDER-030219, POCI-01-0247-FEDER072237. Carlos Fernandes thanks the FCT for the financial support of his work contract through the Scientific Employment Stimulus—Individual Call (10.54499/2021.04016.CEECIND/CP1655/CT0004). Fernando Cagide thanks the FCT for financial support of his work contract (10.54499/DL57/2016/CP1454/CT0001). Bárbara Nunes acknowledges individual PhD fellowship from FCT (10.54499/UI/BD/151088/2021).

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OC13

Investigation of the Cycloheptathiophene-3-carboxyamide Core for the Discovery of Influenza Virus Polymerase inhibitors.

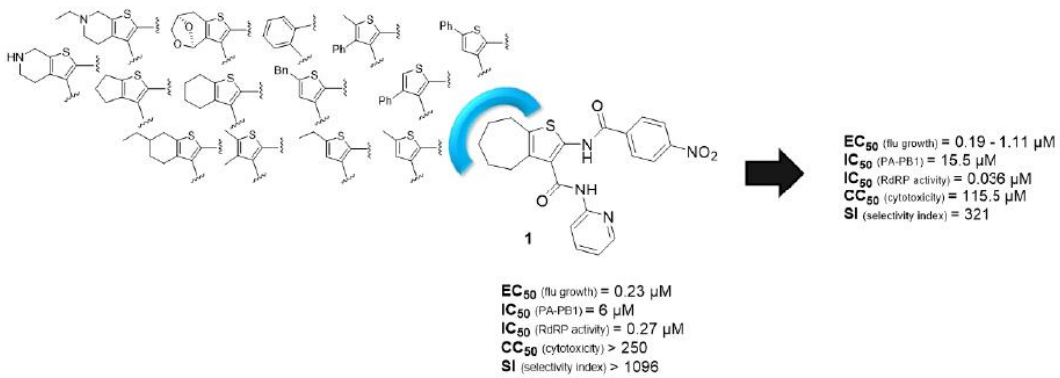
M. Pacetti,[a] M. Casciari,[a] A. Bonomini,[b] C. Bertagnin,[b] T. Felicetti,[a] O. Tabarrini,[b] A. Loregian,[b] S. Massari.[a]

[a] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06123, Perugia, Italy

[b] Department of Molecular Medicine, University of Padua, Via A. Gabelli 63, 35121, Padova, Italy

martina.pacetti@dottorandi.unipg.it

Influenza virus (IV) RNA-dependent RNA polymerase (RdRP) is a well-known and established target for the development of next-generation antivirals. RdRP consists of three subunits: PA, PB1 and PB2, which interact tightly with each other and with various host factors through specific and highly conserved protein-protein interactions (PPIs). It is important to note that only the correct RdRP heterotrimerization allows replication, transcription of the viral genome as well as the evolution of the IV. In addition to inhibiting the catalytic pocket of each subunit, hampering the formation of PPIs is an innovative and promising approach to block RdRP functions and overcome drug resistance, which is the main limitation of approved anti-IV drugs.[1]
Based on compound **1**,[2,3] previously identified by the research group, in this work the attention was focused on the structural exploration of the cycloheptathiophene-3-carboxamide (cHTC) core. Functionalization of the most interesting core obtained resulted in cHTC analogues with improved anti-IV activity against a panel of IV strains coupled with strong RdRP inhibition. In addition, a more efficient and scalable synthesis approach was developed for these promising anti-IV agents.



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OC14

Synthesis and phenotypical discovery of imidazo[2,1-f]triazine ribosides as broad spectrum antitrypanosomal agents

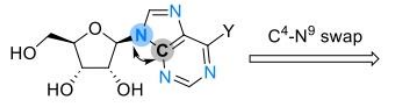
Ewout Van de Velde[a*], Guy Caljon[b], Serge Van Calenbergh[a]

[a] Ghent University, Ottergemsesteenweg 460, 9000 Ghent

[b] University of Antwerp, Universiteitsplein 1, 2610 Wilrijk

*ewout.vandevelde@ugent.be

In 2019, a focused series of purine-like C-nucleosides was synthesized in our lab and evaluated phenotypically against a small panel of protozoa.[1] This study revealed that minimal changes to the nucleobase scaffold may strongly influence activity and host cell toxicity. The purine N^9 – C^4 atom swap in canonical nucleosides resulted in two analogues with low to sub-micromolar broad-spectrum activity against several *Trypanosoma* species at varying toxicities. This incited us to investigate the effect of substituting the canonical NH_2 and OH motifs with various amines, alcohols and thiols.

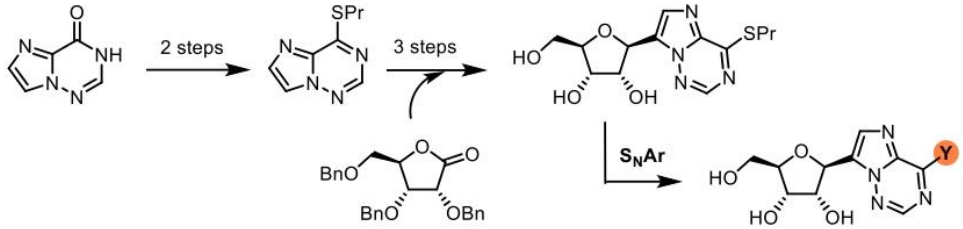


adenosine (ado, Y = NH_2)

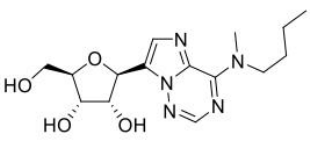
inosine (ino, Y = OH)

	EC_{50} (µM)	CC_{50} (µM)
Y = NH_2	0.25 - 5.15	1.41
Y = OH	0.50 - 3.17	> 64.00

Focusing on feasibility and late-stage diversification, a synthesis method was optimized for variously substituted imidazo[2,1-f]triazine ribosides. The early functionalization of the nucleobase with a thiopropyl group provided ample protection against strongly basic and acidic conditions while also allowing diversification via a direct nucleophilic aromatic substitution reaction (S_NAr) with amines, alcohols and thiols at the very last step, resulting in a very divergent synthesis route.



All analogues were assessed on a cellular level and one displayed EC_{50} values between 73 and 175 nM without any apparent toxicity at 64 µM.



EC_{50} *T. b. brucei*	**0.076 µM**
EC_{50} *T. b. rhod*	**0.073 µM**
EC_{50} *T. cruzi*	**0.120 µM**
EC_{50} *L. infantum*	**0.180 µM**
CC_{50} *MRC-5*	**> 64.00 µM**
CC_{50} *PMM*	**> 64.00 µM**

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OC15

Synthesis of new benzanilide derivatives as HDAC1 selective inhibitors for CRC treatment

Federica Falbo,[a] Gabriele Carullo,[c] Valeria Tudino,[c] Adrian Koch,[b] Sandra Gemma,[c] Stefania Butini,[c] Giuseppe Campiani,[c] Regine Schneider-Stock,[b] and Francesca Aiello[a]

[a] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Ed Polifunzionale, 87036 Rende (CS), Italy

[b] Experimental Tumorpathology, University Hospital, Friedrich-Alexander University Erlangen-Nürnberg, Universitätsstr. 22, 91054 Erlangen, Germany

[c] Department of Biotechnology, Chemistry, and Pharmacy, Via Aldo Moro 2, 53100 Siena, Italy

E-mail: federica.falbo@unical.it

The histone deacetylase (HDAC) family of enzymes has been shown in literature to play a role in colorectal cancer maturation and transformation. Consistent with this role, the expression of several HDACs is upregulated in colon tumors, while compounds that can act as inhibitors of HDACs (HDACi) are potent inducers of growth arrest, differentiation and apoptosis of colon cancer cells *in vitro* and *in vivo* [1]. Several phenolic compounds, such as phenolic acids, have been shown to possess inhibitory activities against HDACs [2] and are, therefore, considered safer alternatives to the currently approved HDACi. This is because a large fraction of these compounds cannot discriminate between different classes of HDACs, and this low selectivity leads to important side effects. In this work, starting from the SAR of known HDACs inhibitors, a library of eighteen phenolic acid derivatives was designed and synthesized to obtain short and long chain benzanilides that can be selective HDAC1 inhibitors. *In silico* preliminary enzymatic assays were evaluated and two compounds (NF3214 and NF3254) seemed to inhibit HDAC1 isoform at very low concentration (micromolar range). *In silico* data were confirmed by in vitro studies. NF3214 and NF3254 were able to inhibit proliferation on a panel of colorectal cancer cell lines (HCT116, HCT116 p21-/-, HT29 and DLD1) in the micromolar range with no toxicity in normal HCEC cells. Cell cycle analysis confirmed apoptosis as cell death mechanism for some cell lines (confirmed by PARP cleavage and p21 induction) while western blot analysis confirmed HDAC1 selectivity. Compounds were also shown to be active *in vivo*, reducing tumors volume in a CAM assay model. These data provide an important starting point for the development of new promising epigenetic modulators specifically targeting HDAC1 for the treatment of colorectal tumors.

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OC16

Rational optimization of the *N*-adamantyl-anthranil amide structural core for the development of new selective ligands for the Cannabinoid subtype 2 Receptor (CB2R)

Annalisa Fanizzi[a], Giovanni Graziano[a], Pietro Delre[b], Jose Brea[c], Alessia Ligresti[d], Chiara Riganti[e], Carmen Abate[a], Mabel Loza[c], Eddy Sotelo[f], Giuseppe Mangiatordi[b], Marialessandra Contino[a], Angela Stefanachi[a] and Francesco Leonetti [a]

[a]Department of Pharmacy-Pharmaceutical Sciences, University of the Studies of Bari "Aldo Moro", 70125, Bari, Italy; [b]CNR – Institute of Crystallography, 70126, Bari, Italy; [c]Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), University of Santiago de Compostela, 15782, Santiago de Compostela, Spain; [d]Institute of Biomolecular Chemistry, National Research Council of Italy, 80078, Pozzuoli, Italy; [e]Department of Oncology, University of Turin, Turin, Italy; [f] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Universidade de Santiago de Compostela, Santiago de Compostela, 15782, Spain.

annalisa.fanizzi@uniba.it

CB2R is a Gi-protein-coupled receptor (GPCR) belonging to the endocannabinoid system (ECS), together with CB1R, endocannabinoids, and related enzymes. CB2R is found in immune tissues and becomes upregulated in microglia cells during pathological states, indicating its role in inflammatory and neurodegenerative diseases[1]. This has increased the efforts focused on developing selective CB2R ligands for their therapeutic potential.

In our study, we aimed to identify new ligands with high affinity and selectivity for CB2R, addressing the need for effective therapeutic agents targeting conditions related to CB2R. We synthesized a series of *N*-adamantyl-anthranil amide derivatives and evaluated their binding affinity and selectivity for CB2R over CB1R[2]. Our design was inspired by the "three-arm pose" of the CB2R antagonist AM10257 in the crystal structure (PDB code:5ZTY). Molecular docking simulations were used to understand the pharmacodynamic profiles, and functional studies were conducted to assess the biological activity of the most promising compounds. Our optimization process underscored the importance of specific structural features for CB2R affinity. Compounds with a phenyl ring or hydrogen on arm 1 and a five-carbon alkyl chain on arm 2 showed the highest affinity. The carboxy-adamantyl amide group on arm 3 was essential for interaction with CB2R. Selected compounds demonstrated significant selectivity for CB2R over CB1R, with promising pharmacodynamic profiles. Functional assays confirmed their potential as CB2R modulators, and molecular docking provided insights into their binding mechanisms. In summary, our study optimized *N*-adamantyl-anthranil amide derivatives, resulting in selective CB2R ligands with potential therapeutic applications for treating inflammatory and neurodegenerative diseases. These findings lay a solid foundation for further development and clinical investigation.

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OC17

Semisynthetic usnic acid-based compounds as promising antifungal agents

Anna Fontana[a], Roberta Listro[a], Valeria Cavalloro[b], Alessio Colleoni[c], Giacomo Rossini[a], Marta E.E. Temporiti[b], Emanuela Martino[b], Simona Collina[a]

[a] Department of Drug Science, University of Pavia (Via Taramelli 12, 27100 Pavia, Italy);
[b] Department of Earth and Environment Sciences, University of Pavia, Pavia, Italy;
[c] Department of Pharmaceutical Sciences, University of Milan, Milan, Italy.
anna.fontana03@universitadipavia.it

In the drug discovery trajectory, nature has always played a key role in the hit identification process either leading to the discovery of biologically active metabolites or inspiring the design of new compounds. Usnic acid (UA) is a well-known secondary metabolite produced by several lichen species which is increasingly emerging for its anti-infective properties. Despite its high therapeutic potentiality in the field of the infectious diseases, UA suffers from poor solubility and systemic uptake along with hepatotoxicity issues.[1] In an attempt to ameliorate the drug-likeness of UA, we conceived the development of novel semisynthetic compounds by properly derivatizing the (*R*)- and (*S*)-UA as enamines with the purpose of evaluating the impact of their chirality on the antifungal activities.[2] All the compounds were assayed against *Candida albicans*, *Candida tropicalis*, and *Trichophyton rubrum* allowing to obtain the first structure-activity relationship clues. Cytocompatibility and water solubility studies were performed for the most promising derivatives which exhibited improved drug-like properties with respect to UA. Taken together, the data collected so far hold promise for the use of the novel compounds in the topical treatment of fungal infections, encouraging further investigations aimed at elucidating their mechanism of action.

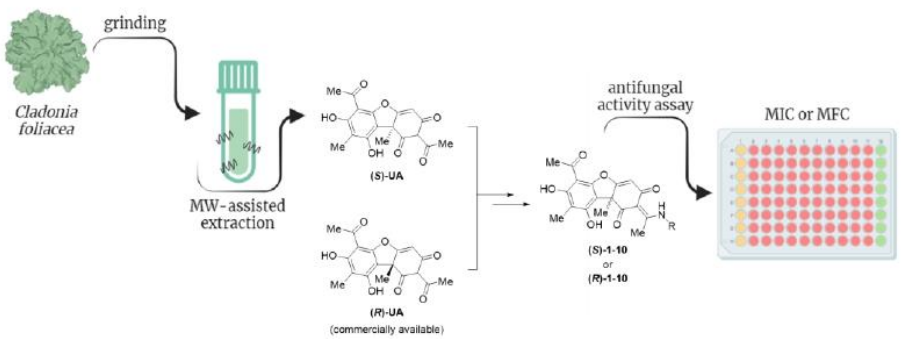

Fig. 1. Representation of the workflow followed to develop the UA-based compounds.

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OC18

Investigating the multi-target potential for complex diseases of the alkaloid-containing pyrrolo[2,1-*a*]isoquinoline scaffold

Rosa Purgatorio,[a] Alisa A. Nevskaya,[b] Tatiana N. Borisova,[b] Angelina Boccarelli,[a] Modesto de Candia,[a] Mauro Niso,[a] Antonio Carrieri,[a] Marco Catto,[a] Orazio Nicolotti,[a] Leonid G. Voskressensky[b] and Cosimo D. Altomare[a]

[a] Department of Pharmacy-Pharmaceutical Sciences, University of Bari Aldo Moro, Via E. Orabona 4, 70125 Bari, Italy

[b] Organic Chemistry Department, RUDN University, 6 Miklukho-Maklaya St, Moscow 117198, Russian Federation

rosa.purgatorio@uniba.it

Neurodegenerative disorders and cancer constitute a public health concern due to the growing incidence and lack in effective therapies. For these multifactorial diseases, the multitarget approach appears as promising strategy for the search of new drug candidates. Diverse isoquinoline alkaloids showed a variety of pharmacological activities, including anticancer and antiviral activities, as well as inhibition of efflux pumps responsible for multidrug resistance (MDR) [1, 2].

In recent years, we synthesized and investigated the medicinal chemistry of several derivatives of 1-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (1-Ph-DHPIQ), that is the scaffold of the alkaloid lamellarins I, which carry out in the C2 position the aldehyde group and related imino adducts, as well as carboxylate groups (COOH, COOEt), nitrile (CN) and Mannich bases (i.e., morpholinomethyl derivatives). Some fifty newly synthesized compounds were assayed for their cytotoxic/antiproliferative activity against diverse cancer cell lines, including some MDR tumors, and against targets related to Alzheimer disease (AD), such as cholinesterases (ChEs), monoamine oxidases (MAOs) and amyloid-β (Aβ) aggregation [3] (Fig. 1). The results from our SAR, in-silico docking and hit-to-lead optimization studies will be presented and discussed.

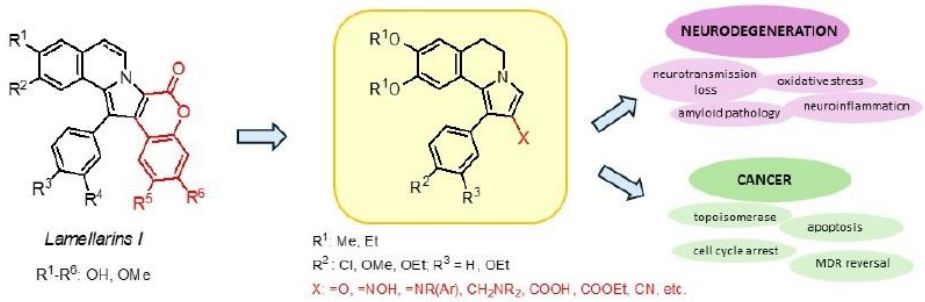

Figure 1. Main targets and biological activities of lamellarins I and synthetic 1Ph-DHPIQ derivatives.

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OC19

Benchmarking a Deep Generative Model for the Optimization of 3-Aroyl-1,4-diarylpyrroles (ARDAP)

Pietro Sciò[a], Romano Silvestri[a], Antonio Coluccia[a], Marianna Bufano[a]

[a] Laboratory Affiliated with the Institute Pasteur Italy–Cenci Bolognetti Foundation, Department of Drug Chemistry and Technologies, Sapienza University of Rome

Piazzale Aldo Moro 5, Roma 00185, Italy

pietro.scio@uniroma1.it

The Molecule Optimization by Reinforcement Learning and Docking (MORLD) method represents a promising deep generative model for the autonomous generation and optimization of lead compounds[1]. By utilizing predicted docking binding affinity as one of the rewards during reinforcement learning, this model does not require any training data. Consequentially, the optimization process becomes dynamically adaptable to any considered target, regardless of the availability of protein-ligand experimental data.

In this study, the source code of MORLD was modified to implement five different docking softwares and a comparative benchmarking was conducted to retrospectively reconstruct the structure-activity relationships (SAR) of 3-aroyl-1,4-diarylpyrroles (ARDAP)[2], a previously reported class of tubulin polymerization inhibitors.

Glide[3] emerged as the best performing software in combination with MORLD, yielding optimization results that closely matched the reported SAR of ARDAP. Interestingly, Glide preemptively excluded a number of largely suboptimal intermediate structures due to incompatibility with its force field[4]. This unexpected behaviour, interpreted by MORLD as an absence of reward, proved highly advantageous for the optimization process by prompting the learning algorithm to concentrate on fewer yet better optimized molecules. Furthermore, by applying a soft restraint to a small portion of the ARDAP scaffold, the optimization process was further enhanced selectively guiding MORLD to improve the binding affinity of the reference binding mode.

Finally, the findings from the retrospective analysis were employed in the rational design of a new ARDAP derivative, which exhibited significant activity against tubulin polymerization and cancerous cell lines.

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OC20

Discovery of a potent steroidal oxime against breast and lung cancers

Ana R. Gomes[1,2*], Elisiário J. Tavares-da-Silva[2], Ana M. Abrantes[1,3,4], Ana C. Gonçalves[3,4,5], Raquel Alves[3,4,5], Maria F. Botelho[1,3,4], Saúl C. Costa[6], Ana S. Pires[1,3,4], Fernanda M.F. Roleira[2]

[1]Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Biophysics Institute of Faculty of Medicine, Azinhaga de Santa Comba, Pólo III - Pólo das Ciências da Saúde, 3000-548 Coimbra, Portugal

[2]Univ Coimbra, CERES, Faculty of Pharmacy, Laboratory of Pharmaceutical Chemistry, Azinhaga de Santa Comba, Pólo III - Pólo das Ciências da Saúde, 3000-548 Coimbra, Portugal

[3]Univ Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal

[4]Clinical Academic Center of Coimbra (CACC), Praceta Professor Mota Pinto, 3004-561 Coimbra, Portugal

[5]Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Laboratory of Oncobiology and Hematology and University Clinics of Hematology and Oncology, Faculty of Medicine

[6]Univ Coimbra, Faculty of Pharmacy, Laboratory of Pharmaceutical Chemistry, Azinhaga de Santa Comba, Pólo III - Pólo das Ciências da Saúde, 3000-548 Coimbra, Portugal

*Email: aprgomes@student.uc.pt

Cancer remains one of the life-threatening diseases worldwide despite the large number of therapeutic approaches currently available. Steroidal compounds possess numerous biological activities being antitumor one of them. Oximes have also been associated with antitumor activity. Thus, we designed and synthesised new steroidal oximes and evaluated their potential antitumor activity against lung and triple-negative breast cancer cells.

Compounds **OX1**, **OX2** and **EP2OX** and their parent compounds **P1**, **OL2**, and **EP2** were synthesized, and their cytotoxicity was evaluated in H1299 (lung) and HCC1806 (breast) cell lines through SRB assay after treatment of the cells with the compounds (1-75 µM). Cell viability, cell death profile, alterations in cell cycle, mitochondrial membrane potential and DNA damage were assessed by flow cytometry.

All compounds decreased H1299 and HCC1806 cancer cell proliferation in a dose-dependent manner, except **OX2**. Overall, the parent compounds decreased cancer cell proliferation in a less pronounced way, proving that the introduction of an oxime group was beneficial for the cytotoxicity displayed. Moreover, the best compound was **EP2OX** in both cell lines with IC_{50} values of 1.13 and 1.95 µM in H1299 and HCC1806, respectively. Further studies with **EP2OX** showed that it was able to decrease cell viability in both cell lines by causing cell death mainly by apoptosis and/or necrosis, which was accompanied by a blockage at phases G_2/M, depending on the cell line and concentration. Furthermore, **EP2OX** induced mitochondrial dysfunction and increased the expression of γH2Ax which, might indicate the presence of double-strand breaks.

Our results show that **EP2OX** possesses a beneficial antitumor effect from the introduction of the oxime group, which is mediated by apoptosis/necrosis and DNA damage, encouraging further studies.

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OC21

Quantitative Composition-Activity Relationships Through Machine Learning Algorithm. Application to Essential Oil Tested as Acetylcholinesterase Inhibitors

Roberta Astolfi[a,*], Luigi Scipione[b], Eleonora Proia[a], Valentina Noemi Madia[b], Davide Ialongo[b] and Rino Ragno[a]

[a]Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University, Rome, Italy.
[b]Department of Drug Chemistry and Technology, Sapienza University, Rome, Italy.

* *Email: roberta.astolfi@uniroma1.it*

Acetylcholinesterase (AChE) is the enzyme responsible for the degradation of acetylcholine (ACh), which is essential for neurological and neuromuscular functions [1]. In Alzheimer's disease (AD) and similar conditions, abnormal AChE expression or low ACh levels require external modulation in order to restore ACh balance [2]. This study experimentally evaluated 59 EOs as AChE modulators and joined with inhibitory data taken from the AI4EssOil database (www.ai4essoil.com) to compile a final dataset of 69 EOs. Machine learning algorithms (RF, GB, SVM, DT, KNN) were used to build a quantitative composition-activity relationships (QCAR) predictive model correlating EOs' chemical compositions with AChE inhibition. The final ML model was obtained with a threshold at 42.7% of AChE inhibition characterized by a dataset splitted into 24 and 45 active and inactive EOs, respectively. Analysis of the QCAR model revealed the key chemical components mostly influencing AChE inhibition to be 1,8-cineole (eucalyptol) carvacrol and 1-nitro-2-phenylethane (NPE). Indeed, eucalyptol was already reported to inhibit AChE, but still its mechanism of action remains unclear [3]. Carvacrol was reported to inhibit AChE and interestingly, its isomer, thymol, was found to be approximately ten times less effective than carvacrol [4]. NPE is one of the main components of *Aniba canelilla* EO, also inhibited AChE and demonstrated synergistic effects if combined with EOs. The NPE/EOs combination reversed memory deficits similarly to donepezil [5]. Molecular docking studies will be performed to investigate NPE and other EOs' components as putative AChE binders and possible fragments to develop new AChE ligands. Further results will be presented.

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OC22

Designing an Effective Pharmacophore Model for Sigma 1 Receptor

Lisa Lombardo,[a] Rosaria Gitto[a], Francesco Ortuso[b], Salvatore Mirabile[a], Stefano Alcaro[b], Agostino Marrazzo[c], Emanuele Amata[c], Laura De Luca[a]

[a]Department of Chibiofaram, University of Messina
Viale Ferdinando Stagno d'Alcontres 31, 98166, Messina, Italy

[b]Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro-Net4Science srl, c/o University "Magna Graecia" of Catanzaro
Viale Europa, 88100, Catanzaro, Italy

[c]Department of Drug Sciences, Medicinal Chemistry Section, University of Catania
Viale A Doria 6, 95125 Catania, Italy

lisa.lombardo@studenti.unime.it

The Sigma 1 Receptor (S1R) functions as a chaperone protein, regulating numerous physiological pathways. Its multifaceted role within cells has identified S1R as a promising therapeutic target in drug discovery, particularly for central nervous system (CNS) diseases and cancers.[1] It is well-established that a variety of chemical entities can interact with S1R, but the literature highlights three key pharmacophore features: two hydrophobic regions and a central basic core.[1] In this work, a comprehensive analysis of crystallographic structures of human S1R in complex with various ligands was carried out to derive a reliable pharmacophore model. Firstly, molecular dynamics simulations were employed to generate an ensemble of conformations for each complex. Subsequently, trajectory frames were submitted to the Grid-Based Pharmacophore Model method to identify the critical interactions between ligands and residues.[2] The resulting data were used as input to build a shared pharmacophore model. The performance of the hypothesis was assessed through theoretical and experimental validations. Corroborating the predictiveness of our S1R pharmacophore, a set of compounds was designed, synthesized, and evaluated through *in vitro* S1R binding assays. The optimal K_i affinity values (ranging from 4.8 to 25.4 nM) further supported the robustness of our computational approach. Hence, the proposed pharmacophore model could be a valuable tool to design/discover new S1R ligands.

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OC23

Revealing Polyphenols' Therapeutic Potential in Multiple Myeloma: Insights from Computational Studies on 20S Proteasome

Emanuela Marchese[a], Francesca Alessandra Ambrosio[a], Nicola Amodio[b], Stefano Alcaro[a,c,d], Giosuè Costa[a,c]

[a] Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100 Catanzaro, Italy

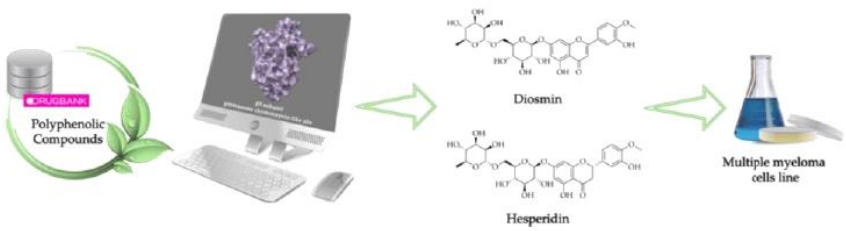
[b] Dipartimento di Medicina Sperimentale e Clinica, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100 Catanzaro, Italy

[c] Net4Science Academic Spin-Off, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100 Catanzaro, Italy

[d] Associazione CRISEA—Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Loc. Condoleo, 88055 Belcastro, Italy

e.marchese@unicz.it

Polyphenols are natural compounds found in plant-based foods. They have several biological activities and can protect against various physiopathological conditions, including cancer. This study investigates the potential mechanisms of action of polyphenols, specifically their effect on the proteasome. The proteasome is an attractive therapeutic target in cancers such as multiple myeloma. Starting from the DrugBank database as a repository of FDA-approved polyphenolic molecules, a structure-based virtual screening (SBVS) study was conducted through the Schrodinger Suite [1]. From 86 polyphenolic compounds, 2 promising candidates, Hesperidin and Diosmin, were selected based on their theoretical binding affinity and interactions with key residues of the chymotrypsin binding site. The additional evaluation of the biological activity revealed that these two compounds can inhibit β5-proteasome activity and exhibit anti-tumor effects against proteasome inhibitor-sensitive or resistant multiple myeloma cell lines [2].



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OC24

Scalable microfluidic method for tunable liposomal production by a rational approach

Giorgio Buttitta[a], Simone Bonacorsi[a,b], Chiara Barbarito[b], Mauro Moliterno[b], Simona Pompei[b], Gabriele Saito[b], Irene Oddone[b], Giuliana Verdone[b], Daniela Secci[a], Sergio Raimondi[b]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.
[b] BSP Pharmaceuticals S.p.A., Via Appia Km. 65,561, 04013 Latina Scalo (LT), Italy.

giorgio.buttitta@uniroma1.it

Liposomes are sphere-shaped vesicles formed by a phospholipid bilayer (unilamellar liposomes) and/or a concentric series of multiple bilayers (multilamellar liposomes) enclosing a central aqueous core.[1] Currently, there are two main groups of strategies to fabricate liposomes. First, top-down approaches that involve the formation of large vesicles, followed by size reduction techniques (extrusion, microfluidization, high-shear mixing, sonication) to attain desired size and lamellarity. Second, bottom-up methods, which promote the formation of small vesicles from individual lipid monomers.[2] Size, dispersity and lamellarity of liposomes may all vary depending on the method used. Among the bottom-up methods, a promising approach for liposome production is based on the microfluidic technology. Overall, the application of microfluidics to liposome fabrication consists in forcing a stream of organic solution of lipids to flow into the inner channel of the device. The organic stream is intersected by one or multiple streams of a water-based solution. The mixing of the two phases causes a change in the polarity of the solvent, causing a decrease in the solubility of the lipids, that triggers the formation of liposomes by a mechanism known as "self-assembly".[3]
Different parameters can affect the microfluidic liposomal formation: the lipid composition and their intrinsic features (charge and phase transition temperature), the chip architecture (inlet geometry, mixing microchannel cross section, etc.) and the mixing temperature.[4]
In this study an off-the-shelf microfluidic system and a methodological approach are presented for the optimization, validation and scale-up of highly monodisperse liposomes manufacturing. Starting from a Doxil®-like formulation (HSPC, MPEG-DSPE and cholesterol), a rational approach (Design of Experiments) was applied for the screening of the process parameters affecting the quality attributes of the product (mainly size and polydispersity). Additional DoEs were conducted to determine the effect of critical process parameters (cholesterol concentration, total flow rate TFR and flow rate ratio FRR), thus assessing the formulation and process robustness. A scale-up was then successfully accomplished. The procedure was applied to a Marqibo®-like formulation as well (sphingomyelin and cholesterol) to show the generality of the proposed formulation, process development and scale-up approach. The application of the system and method herein presented enables the large-scale manufacturing of liposomes, in compliance with the internationally recognized regulatory standards for pharmaceutical development (Quality by Design).

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OC25

Natural F-series Pyrrolomycins: Microwave-assisted total synthesis, anticancer activity, and mechanism of action

Ignazio Sardo [a], Marilia Barreca [a], Miriam Buttacavoli [a], Gianluca Di Cara [a], Cesare D'Amico [a], Emanuela Peri [a], Virginia Spanò [a], Giovanna Li Petri [b], Paola Barraja [a], Patrizia Cancemi [a], Alessandra Montalbano [a], Maria Valeria Raimondi [a]

[a] Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy

[b] Institute of nanostructured materials (ISMN), CNR, via U. La Malfa 153, 90146 Palermo, Italy

ignazio.sardo@unipa.it

Pyrrolomycins (PMs) are a class of polyhalogenated small natural antibiotics. Their antibacterial activity has been extensively studied as inhibitors of Sortase A in Gram-positive bacteria (e.g *S. aureus*) [1]. Moreover, they act as protonophores thus decoupling oxidative phosphorylation in Gram-positive and Gram-negative bacteria [2]. Although the antitumour effect of a class of pyrrolomycin-based derivatives was reported [3], overall this class of compounds is poorly investigated. Thus, we focused on the synthesis of natural F-series PMs to investigate their antiproliferative effect. Total synthesis of F-series PMs was performed by MAOS technique and their cytotoxic activity on HCT116 and MCF-7 cancer cell lines was evaluated and compared to the effects on hTERT-RPE-1 nontumoral epithelial cell line. Morphological abnormalities were observed, the expression level of markers related to survival pathways, autophagic cell death and cytoskeletal proteins were also analyzed by Western blot analysis. F-series PMs showed anticancer activity at submicromolar level (IC_{50} ranged from 0.35±0.1 µM in HCT116 to 1.21±0.3 µM in MCF-7) with minimal effects on hTERT-RPE-1. Interestingly, they produced several morphological changes at cellular level suggesting that F-series PMs induce non-conventional cell death and impair cytoskeleton organization. Moreover, the exposure to tested compounds induced a remarkable reactive oxygen species (ROS) increase, suggesting an important role of ROS in PMs toxicity. F-series PMs can be considered as promising antitumoral drugs not only for their biological activity but even more importantly for their non-canonical mechanism of action on the cell membranes and cytoskeleton.

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OC26

Progresses in targeting mono-ADP ribosylating enzymes: towards selective inhibitors

Chiara Sarnari,[a] Maria Giulia Nizi,[a] Juho Alaviuhkola,[b] Serena Massari,[a] Giuseppe Manfroni,[a] a Lari Lehtiö,[b] and Oriana Tabarrini[a]

[a] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06123, Perugia, Italy

[b] Faculty of Biochemistry and Molecular Medicine& Biocenter Oulu, University of Oulu, Pentti Kaiteran katu 1, 5400 Oulu, Finland

chiara.sarnari@dottorandi.unipg.it

Mono-ADP ribosylating enzymes (mono-ARTs), a subfamily within the PARP family, play pivotal roles in diverse cellular processes, including cell replication, immune response, apoptosis, and neurodevelopment. Despite their significant biological functions and therapeutic potential in treating not only cancer but also inflammation and neurological disorders, the development of selective mono-ARTs inhibitors remains challenging. One of the major difficulties, which is common to all PARPs inhibitors, relies in the highly conserved nature of the NAD+ binding pocket across all PARP subfamilies, and most of the inhibitors work as NAD+ mimetics. Additionally, a limited understanding of the subtle structural differences between mono-ARTs further hinders the design of highly selective inhibitors.[1]

We have recently reached the goal to achieve selectivity by exploring innovative heterocycles as new nicotinamide mimetic scaffolds.[2,3] In particular, by investigating the [1,2,4]triazolo[3,4-b]benzothiazole (TBT) core, compound OUL232[2] emerged as the most potent PARP10 and PARP15 inhibitor described to date and the sole PARP12 inhibitor ever reported. Based on this valid starting point, a wide SAR exploration has been performed with the aim to increase even more both the potency and selectivity while inhibiting other specific enzymes within the mono-ARTs subfamily. Thus, based on previous biological results along with the co-crystallographic structures, new derivatives were designed and synthesized. Seeking for specificity, various substituents were placed in distinct positions of the scaffold to reach some less conserved regions (e.g. D-loop or mono-ARTs hydrophobic cavity) of the PARPs catalytic domain. Furthermore, to contribute to clarifying the physiopathological roles of mono-ARTs in cellular contexts, the TBT-based compounds were also elaborated in specific mono-ARTs degraders. All the synthesized compounds were tested against a wide panel of PARPs, from which interesting derivatives with new SAR insights emerged, that will be the object of the presentation.

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OC27

Photodynamic therapy for endometrial cancer: can aldehyde dehydrogenase play a key role in eliminating cancer stem cells?

Beatriz Serambeque[a,b], Sofia Lopes[a,b], Gabriela Correia-Barros[a,b], Margarida Silva[a,b], Bruna Costa[a,b,c], Marta Piñeiro[c], Nelson AM Pereira[c], Ana Cristina Gonçalves[b,d,e], Raquel Alves[b,d,e], Maria Filomena Botelho[a,b,d], Teresa MVD Pinho e Melo[c], Maria João Carvalho[a,b,d,f,g] and Mafalda Laranjo[a,b,d]

[a] Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Institute of Biophysics, Faculty of Medicine, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal; [b] Univ Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal; [c] Univ Coimbra, Coimbra Chemistry Centre - Institute of Molecular Sciences and Department of Chemistry, Rua Larga, 3004-535 Coimbra, Portugal; [d] Clinical Academic Centre of Coimbra (CACC), Praceta Professor Mota Pinto, 3004-561 Coimbra, Portugal; [e] Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Laboratory of Oncobiology and Hematology (LOH) and University Clinics of Hematology and Oncology, Faculty of Medicine, 3000-548 Coimbra, Portugal; [f] Univ Coimbra, Universitary Clinic of Gynecology, Faculty of Medicine, 3000-548 Coimbra, Portugal; [g] Department of Gynecology, Obstetrics, Reproduction and Neonatology, Unidade Local de Saúde de Coimbra, Praceta Professor Mota Pinto, 3004-561 Coimbra, Portugal

beatriz.serambeque@student.uc.pt

In endometrial cancer (EC), inhibition of aldehyde dehydrogenase (ALDH) suggests that this enzyme could play a relevant role in stemness pathways. To explore its potential as a therapeutic target, our research team proposed a novel therapy based on photodynamic therapy using 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused chlorins (PX-PDT), developing a cancer stem cells (CSC)-targeted PX-PDT (A-PX-PDT) with functionalised aldehyde moieties (A-PX-PDT). ECC-1, RL95-2, and HEC-1-A were exposed to 0.1 and 1 µM dihydroxymethyl (PX1), dicarboxylic acid (PX3), and dimethylesther (PX4) for 24 hours, followed by light activation (7.5mW/cm^2, 10J). Flow cytometry and an indirect strategy with ROS scavengers assessed the viability and PDT-induced cell death types, cell cycle, and pivotal ROS, respectively. Post-PDT, we also evaluated ALDH expression through Western blot. To determine the colocalisation of PX1, EC cells were incubated with 0.5 µM and analysed using confocal microscopy. To assess the efficacy of PDT on endometrial CSC, a sphere-forming protocol was performed, and spheres were treated with PX- and three targeted PX, a dialdehyde (A-PX1), and two mono-aldehydes (A-PX2/3; 0.5 – 10 µM). Cell viability and metabolic activity upon PDT were assessed using luminescence and colourimetry assays and confirmed with confocal microscopy. Our findings revealed that PX-PDT significantly decreased EC cell viability, mainly inducing death by late apoptosis/necrosis, being the oxygen singlet, the most important ROS in photodynamic action. PDT also influenced the cell cycle, increasing the subG0/G1 and G0/G1 phases and decreasing the S phase. Moreover, PX-PDT appears to induce a decrease in the ALDH expression. PX1 tended to accumulate into cytoplasmic organelles and plasma membrane without reaching the nucleus. Regarding endometrial CSC, Px-PDT decreased CSC metabolic activity, presenting a concentration-dependent behaviour. A similar result seems to be observed with A-PX-PDT, which also affects CSC viability. In conclusion, Px-PDT appears to be an effective approach to treating EC, endorsing an endometrial CSC-targeted PDT strategy. FCT supports the CIBB through the Strategic Projects (DOI: 10.54499/UIDB/04539/2020 and DOI: 10.54499/UIDP/04539/2020) and the Associated Laboratory funding LA/P/0058/2020 (DOI: 10.54499/LA/P/0058/2020). FCT supports CQC through the Strategic Projects (DOI: 10.54499/UIDB/00313/2020 and DOI: 10.54499/UIDP/00313/2020) and PTDC/QUI-QOR/0103/2021 (FCT, I.P./MCTES, PIDDAC). PhD Scholarship from FCT and European Social Fund to Beatriz Serambeque (10.54499/2020.07672.BD) and Bruna Costa (2022.12013.BD).

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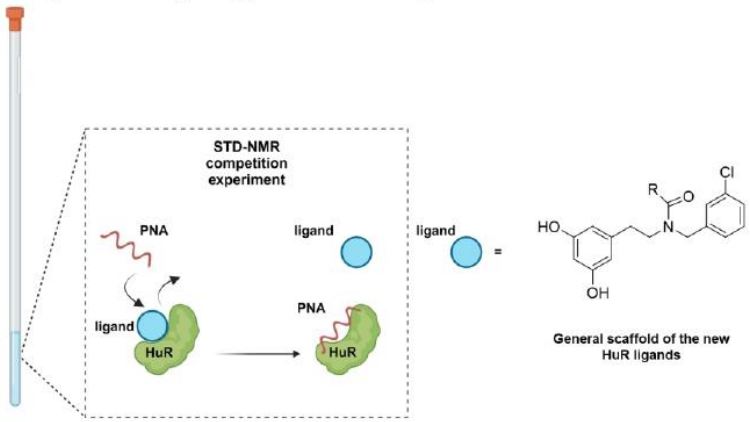
OC28

Discovery of SMol acting on HuR-RNA complexes. STD-NMR competition studies using Peptide Nucleic Acids

Martina Garbagnoli,[a] Roberta Listro,[a] Irene Gado,[c] Pasquale Linciano,[a] Daniela Rossi,[a] Francesca Ambrosio,[b] Giosuè Costa,[b] Francesca Vasile,[c] Simona Collina[a]
[a] Department of Drug Sciences, University of Pavia
[b] Net4Science Academin Sin-off, University "Magna Graecia" of Catanzaro, Catanzaro, Italy
[c] Department of Chemistry, University of Milan, Milan, Italy
Viale Taramelli 12, 27100, Pavia, Italy
martina.garbagnoli01@universitadipavia.it

RNA-binding proteins (RBPs) play a key role in regulating RNA stability, RNA fate and function, gene expression, post-transcriptional modifications, and cellular activities.[1] Among the various RBPs identified to date, the Hu proteins are the most extensively studied. Specifically, HuR influences several cellular processes, including cell proliferation, differentiation, and stress response, and is frequently overexpressed in various solid tumors.[2] In this work, a Peptide Nucleic Acid (PNA) fragment was used to form the complex with HuR instead of RNA, and such a complex was studied by molecular modeling and STD-NMR. Indeed, PNA overcomes the limitations of using RNA due to its instability to environmental RNases. Successively, STD-NMR was used to evaluate the ability of HuR ligands to interfere with HuR-PNA complex. The experiments were supported by molecular modelling studies. Thus, we propose an innovative use of PNA as tools to analyze interaction and interfering properties of compounds targeting RBP-RNA complexes.



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OC29

Drug discovery of natural agents endowed with anticancer properties

Adriana Gargano,[a,b] Stefano Alcaro[a,b,c]

[a] Dipartimento di Scienze della Salute, Università "Magna Graecia" di Catanzaro, Campus Universitario "S. Venuta", Viale Europa 88100, Catanzaro, Italy

[b] Associazione CRISEA – Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Località Condoleo, 88055 Belcastro (CZ), Italy

[c] Net4Science Academic Spinoff, Università "Magna Græcia" di Catanzaro, Campus Universitario "S. Venuta", Viale Europa, 88100 Catanzaro, Italy

a.gargano@unicz.it

In recent years there has been a significant focus on the development of Plant-Based Medicines, crediting their potential as natural remedies against so many diseases. Starting from this premise, the aim of my doctoral project is the identification potential anticancer agents of natural origin, so as to design a botanical garden enriched with these plants endowed with pharmaceutical and nutraceutical properties.

Specifically, my research has been based on two different approaches: Target-based Drug Discovery (TDD) and Phenotypic Drug Discovery (PDD).

The TDD approach, through the development of a pharmacophoric hypothesis, shape screening, and virtual screening, has enabled the identification of natural compounds potentially active on several targets involved in tumor development and progression, such as CK1ε, HDAC8, IL-20R. For each of them, enzymatic and cell proliferation assays are ongoing.

During my internship carried out at the Instituto de Bio-Orgánica "Antonio González" (Tenerife, Spain), the anticancer activity of different molecules extracted from plants endemic to the South American territory was evaluated both in monolayers and in 3D cell cultures (spheroids). Since the approach in this case was PDD, target fishing studies are ongoing so as to identify the targets responsible for this anticancer activity.

In addition, this close collaboration with the team of my foreign supervisor, Prof. José Manuel Padrón, and with several international universities, led to the publication of an important scientific work involving the conjugation of the natural coumarin moiety with delocalized lipophilic cations as selective carbonic anhydrase inhibitors. In this context, my molecular docking and molecular dynamics studies have allowed us to elucidate their mechanism of action.[1]

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OC30

Targeting TERRA G-quadruplex: Design and Biophysical Evaluation of Chromene Scaffold Derivatives

Roberta Rocca,[a,b] Anna Artese[a,b], Alessandra Gianoncelli[c], Giovanni Ribaudo[c], Sara Richter[d], PierFrancesco Tassone[e] and Stefano Alcaro[a,b]

[a] *Dipartimento di Scienza della Salute, Università "Magna Græcia" di Catanzaro, 88100 Catanzaro, Italia*
[b] *Net4science Srl, Università "Magna Græcia" di Catanzaro, 88100 Catanzaro, Italia*
[c] *Department of Molecular and Translational Medicine, University of Brescia, 25121 Brescia, Italy*
[d] *Department of Molecular Medicine, University of Padua, Italy*
[e] *Department of Experimental and Clinical Medicine, Magna Græcia University, Catanzaro, Italy*

rocca@unicz.it

The telomeric repeat-containing RNA (TERRA) plays a critical role in telomere biology and genomic stability, making it an attractive target for anticancer therapy. Recent research has highlighted the potential of chromene-based compounds in stabilizing TERRA G-quadruplexes (G4s), offering a novel therapeutic approach against multiple myeloma [1,2]. Here, we employed a rational drug design strategy to develop chromene derivatives as potential ligands for TERRA G4s. Through structural modifications and molecular modeling, we screened a compound library focused on the chromene scaffold via docking simulations to evaluate the binding affinity to TERRA G4 structures [3]. Biophysical assessments, including mass spectrometry and circular dichroism spectroscopy, confirmed the binding affinity and selectivity of these derivatives for TERRA G4s. These findings highlight the potential of chromene derivatives as lead candidates for therapeutics targeting TERRA-related diseases, such as cancer and aging-associated disorders.

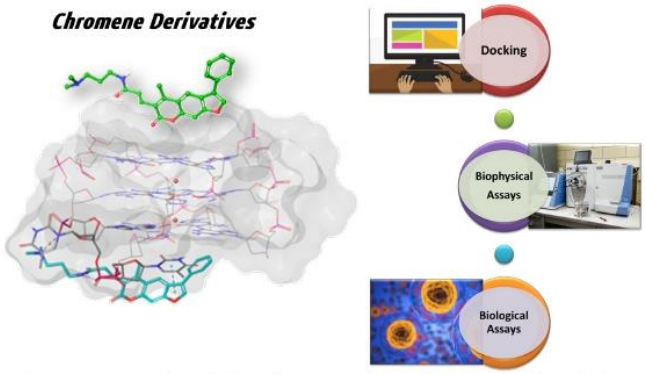

Figure 1 – 3D structure of TERRA complex with a chromene-based compound and the workflow applied for the discovery of new chromene derivatives.

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OC31

Lithospermic Acid: Unveiling Its Potential as a Pancreatic Lipase and hCA V Inhibitor through In Silico and In Vitro Studies

Emanuele Liborio Citriniti[1], Roberta Rocca[1,2,3], Giosuè Costa[1,2],Claudia Sciacca[4], Nunzio Cardullo[4], Vera Muccilli[4], Anastasia Karioti[5], Fabrizio Carta[6], Claudiu T. Supuran[6], Stefano Alcaro[1,2,3], Francesco Ortuso[1,2]

[a 1] Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Viale Europa, 88100 Catanzaro, Italy;
[2] Net4Science S.r.l., Università "Magna Græcia" di Catanzaro, Viale Europa, 88100 Catanzaro, Italy;
[3] Associazione CRISEA—Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Località Condoleo di Belcastro, 88055 Catanzaro, Italy;
[4] Dipartimento di Scienze Chimiche, università degli Studi di Catania. V.le A. Doria 6, 95125 Catania
[5] Laboratory of Pharmacognosy, School of Pharmacy, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece;
[6] NEUROFARBA Department, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, 50019 Florence, Italy;

emanueleliborio.citriniti@unicz.it

Obesity is a medical condition characterized by the excessive accumulation of body fat, leading to adverse health effects such as chronic inflammation, dyslipidemia, cardiovascular dysfunctions, and certain types of cancer. [1] Among the enzymes involved in obesity, mitochondrial human Carbonic Anhydrase VA (*h*CA VA) and Pancreatic Lipase (PL) are promising pharmacological targets. In this study, we present the discovery and detailed analysis of lithospermic acid (LA) as a promising therapeutic candidate. [2-3] Using a structure-based computational approach (SBVS), we predicted the binding affinity and stability of LA towards *h*CA VA and PL. Advanced techniques such as molecular docking and molecular dynamics simulations (MDs) indicated a promising in *silico* affinity of LA towards both targets. Subsequent in vitro studies confirmed these predictions, demonstrating that LA inhibits both target enzymes in the micromolar range. Our results suggest that lithospermic acid is a promising natural dual inhibitor with a potential applications in the innovative anti-obesity pharmacological strategies.

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PEA-1

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University:
University of Catanzaro, "MAGNA GRECIA", Catanzaro, Italy

Supervisor:
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Doctoral thesis:
Development of multi-target agents with anti-cancer activity

PEA-2

Dr. Agnieszka OLEJARZ-MACIEJ

University:
Jagiellonian University Medical College, Kraków, Poland

Supervisor:
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Doctoral thesis:
Multidirectional activity of new histamine and adenosine receptor ligands"

PEA-3

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Doctoral thesis:
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PEA-4

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Doctoral thesis:
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PEA-5

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Supervisor:
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Doctoral thesis:
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PEA-6

Dr. Piotr STEPNICKI

University:
Medical University of Lublin, Poland

Supervisor:
Prof. Agnieszka Kaczor, Prof. Agata Bartyzel

Doctoral thesis:
Optimization of virtual hits D2AAK3 and D2AAK2 in the search for new drugs for the treatment of schizophrenia

Flash poster presentation

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FP1

Theragnostic probes targeting matrix metalloproteinases 2 and 9

Lourdes Acosta, José María Zapico, Irene Ortín, Alba Gil-Rivas, Beatriz de Pascual-Teresa, Ana Ramos

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

E-mail: l.acosta1@usp.ceu.es

Keywords: Matrix metalloproteinase 2, Matrix metalloproteinase 9, fluorescent probes

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases with the ability to break down connective tissue [1]. The level of MMP-2 and MMP-9 can be used as a tumour marker since it is associated to cancer progression and they are overexpressed in different types of tumours such as, bladder, breast, colon, lung, prostate, and gastric cancers [2,3].

In the present work, we combine different fluorescent probes with a series of MMPs inhibitors. These inhibitors are conformed by a hydroxamic acid that acts as zinc-binding group (ZBG), capable of coordinating the Zn^{2+} present in the active site and, a subunit designed to interact with the S1´ pocket responsible for the selectivity between the different types of MMPs [4,5].

A new series of compounds was synthetized by connecting through a linker different fluorescent probes with known MMPs inhibitors. Then, it was validated that those modifications did not compromise the affinity for MMP-2/9, by the in vitro evaluation of their theragnostic properties.

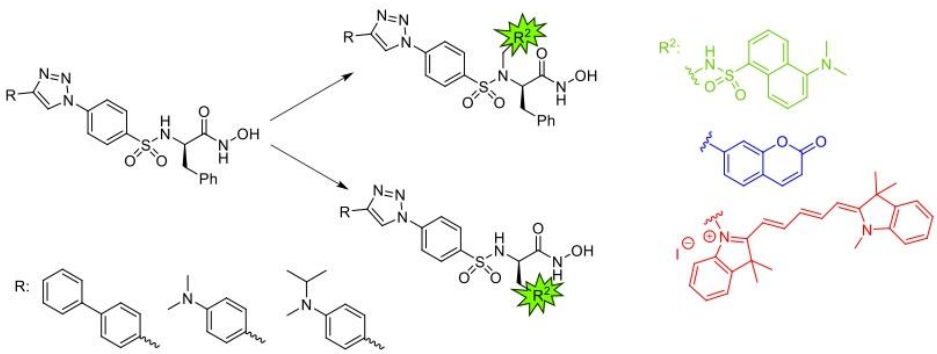

Figure 1. Design of potential MMP-2/9 fluorescent probes

Financial support from PID2021-123786OB-I00 *(MICIU/FEDER, UE) is kindly acknowledged. L.A is supported by PEJ-2020-AI/BMD-17635 (CAM research support).*

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FP2

Beyond The Rule of 5 – synthesis and *in vitro* evaluation of 5-HT$_7$ receptor antagonists with "molecular obesity"

Patryk Pyka[a,b], Sabina Podlewska[c], Sabrina Garbo[d], Grzegorz Satała[c], Katarzyna Kucwaj-Brysz[a], Cecilia Battistelli[d], Ewa Szymańska[a], Jadwiga Handzlik[a]

[a] *Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland*

[b] *Doctoral School of Medical and Health Sciences, Jagiellonian University Medical College, 31-530 Kraków, Poland*

[c] *Maj Institute of Pharmacology Polish Academy of Sciences, Smetna 12, 31-343 Kraków, Poland*

[d] *Istituto Pasteur Italia, Fondazione Cenci-Bolognetti, Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161, Rome, Italy*

patryk98.pyka@doctoral.uj.edu.pl

The 5-HT$_7$ receptor (5-HT$_7$R) is a serotonin receptor that plays a crucial role in regulating various physiological and pathological processes[1]. Research has shown that selective 5-HT$_7$R antagonists have potential therapeutic effects in reducing depressive symptoms and improving REM sleep, making this protein an attractive target for the development of new treatments for depression and other psychiatric disorders[2]. Despite the development of several 5-HT$_7$R ligands, none have successfully completed clinical trials due to issues related to various ADMET properties[3].

In this study we investigated the impact of molecular weight (MW) on the activity and ADMET properties of 5-HT$_7$R ligands (Fig. 1), particularly exploring the potential benefits of "molecular obesity" in discovering new drug candidates with enhanced receptor activity and selectivity.

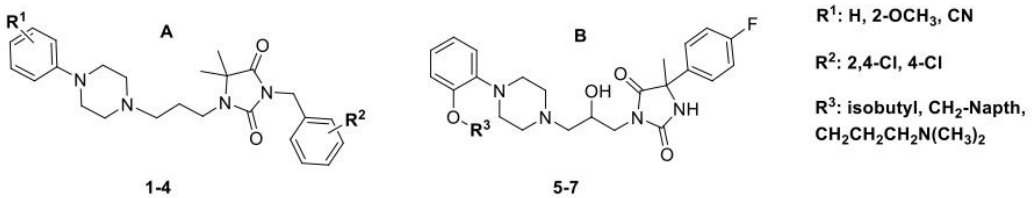

Fig. 1. Structures of the obtained compounds divided into two chemotypes A and B.

The compounds were obtained in a multistep synthesis and assessed in *in vitro* studies for 5-HT$_7$R affinity and selectivity over off-targets, followed by complete ADMET profile evaluation. Additionally we investigated the effect of chosen active compounds (**5-7**) on the expression of certain genes in SH-SY5Y cells. Compound **7** showed a promising ADMET profile and high selectivity over other 5-HTR subtypes, making it a strong candidate for further testing and optimization.

Partially supported by National Science Centre grant no. 2023/49/N/NZ7/02144 and 2018/31/B/NZ2/00165

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FP3

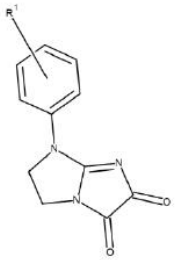
In search of tyrosinase inhibitors with chelating properties

Barbara Bogdańska [1,2], ***Dariusz Matosiuk*** [2]

[1] *Medical University of Lublin, Doctoral School*
[2] *Chair and Department of Drug Synthesis and Chemical Technology, Medical University of Lublin*
W. Chodźki 4a, 20-093 Lublin,
email:barbara.bogdanska@umlub.pl; dariusz.matosiuk@umlub.pl

Skin hyperpigmentation affects a large group of the human population and is a challenge for cosmetologists and dermatologists. The accumulation of overproduced pigment in the skin may cause discoloration problems, including freckles, age spots, and skin diseases, such as melanoma. The process of melanin synthesis is called melanogenesis, and takes place in melanocytes, special cells in the basal layer of the dermis. One of the most important enzymes in this process is tyrosinase. It is a copper-containing enzyme belonging to the family of oxygen oxidoreductases (type 3 metalloprotein) and has two copper atoms and an active oxygen atom at the active site.

Classic tyrosinase inhibitors come from both natural and synthetic sources. Natural tyrosinase inhibitors such as flavonols (e.g. campferol), coumarins (e.g. aloesin), aldehydes (e.g. cinnamaldehyde, cuminaldehyde) mostly come from plants. Kojic acid and azelaic acid are fungal metabolites. Synthetic compounds (e.g. diones or thiosemicarbazides) are also tyrosinase inhibitors. Their research is becoming increasingly important due to their skin whitening effects. Unfortunately, due to the toxicity of most inhibitors, they cannot be used orally or applied to the skin for a long period of time. Kojic acid, a known ingredient in skin lightening cosmetics, has been found to be safe for human skin at concentrations up to 1%, but a depigmenting effect has been observed at 4% concentrations. As a result, it is necessary to search for new derivatives with higher selectivity, potency and lower toxicity.



Our goal was to obtain 1-arylimidazo[1,2-a]imidazole-5,6-dione oximes as compounds with strong chelating properties. The synthesis began with the preparation of N-arylethyldiamines, which were converted from 2-aminoimidazoline borohydrides in reaction with cyanogen bromide and further to imidazo[1,2-a]imidazole-5,6-diones in reaction with diethyl oxalate. 6-hydroxyimine derivatives were obtained by reaction with hydroxylamine. The ethylenediamines necessary for the synthesis were obtained in the alkylation reaction with aziridine or 2-bromoethylamine hydrobromide.

Rys. 1 General oxime structure

LITERATURE

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FP4

Estimation of human serum albumin binding of N-hydroxyurea derivatives of flurbiprofen and diclofenac using a high-performance liquid chromatography method

Jelena Savić, Jelena Bošković, Olivera Čudina, Vladimir Dobričić

Department of Pharmaceutical Chemistry, Faculty of Pharmacy - University of Belgrade

Vojvode Stepe 450, Belgrade, Serbia

jelena.boskovic@pharmacy.bg.ac.rs

Human serum albumin (HSA) plays an important role in transporting drugs, particularly affecting their distribution and elimination rates. Two dual COX-2 and 5-LOX inhibitors (compounds **2** and **3**) were designed by combining pharmacophores of two non-steroidal anti-inflammatory drugs (flurbiprofen and diclofenac, respectively) and a 5-LOX inhibitor zileuton (Figure 1) [1]. In order to estimate their binding to the HSA, retention behavior was tested on CHIRALPAK® HSA chromatographic column (100×4 mm, 5 µm particle size). The mobile phase consisted of 10 mM ammonium acetate and acetonitrile (85/15, v/v). The mobile phase flow rate was 0.9 mL/min, while column temperature was 25°C. Flurbiprofen and diclofenac had the highest retention factors (k = 11.08 and k > 199), indicating the strongest binding to the HSA. Zileuton had the lowest k value (2.65). Compounds **2** and **3** had lower k values in comparison to flurbiprofen and diclofenac (k = 7.49 and k = 8.28), indicating that the introduction of N-hydroxyurea group of zileuton weakens interactions with HSA.

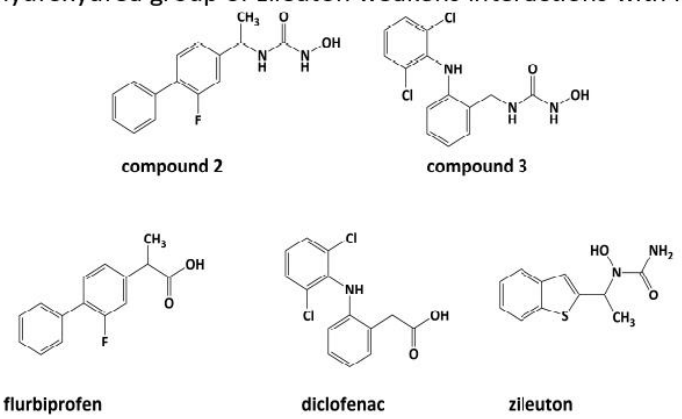

Figure 1. Chemical structures of tested compounds

Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, 7739840, Utilization of interplay between inflammation and cancer in the development of compounds with anticancer activity – INFCANPLAY.

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FP5

Discovery of an allosteric binding site for small molecules in Gs protein

Laura Carosella,[a] Stefano Raniolo,[a] Federica Santoro,[b]

Diego Brancaccio,[b] Vittorio Limongelli[a]

[a]Faculty of Biomedical Sciences, Universita' della Svizzera Italiana

Via la Santa 1, 6962 Lugano, Switzerland

[b]Department of Pharmacy, University of Naples "Federico II"

Via Domenico Montesano 49, 80131 Napoli, Italy

laura.carosella@usi.ch

G proteins are heterotrimeric complexes comprised of α, β, and γ subunits. G proteins are typically categorized into four major families – G_s, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$ – based on the homology of the Gα subunit.[1] They are extensively investigated as intracellular effector proteins of G Protein Coupled Receptors (GPCRs), a pharmacologically relevant membrane protein family targeted by one-third of marketed drugs.[1,2] Historically, drug design studies focus on the extracellular orthosteric binding site of GPCRs to develop new medications. However, the so identified drugs might present adverse effect due to the lack of selectivity in the recruitment of one intracellular effector over the others (e.g., G proteins over beta-arrestins or one specific G protein subtype over the others).[3,4] In this background, we have decided to investigate the Gs protein with the scope of identifying a putative small molecule binding site that could be targeted to discover ligands capable of modulating the GPCR/G protein interaction from the intracellular side. In detail, we conducted molecular simulations on Gs protein, pinpointing an allosteric binding site situated in close proximity with the α5 helix and the C-terminal region, which are involved in its binding interaction with GPCRs. Preliminary studies revealed that this pocket exhibited promising druggability characteristics, including optimal volume, polarity, and the presence of residues not conserved across various G protein subtypes. This finding prompted us to target the newly discovered binding site using docking-based virtual screening calculations to identify the first G protein allosteric ligands. In the present poster, I report the procedure leading to the identification of the G protein allosteric binding site and the results of the virtual screening campaign that enabled to identify the compounds with the best binding affinities among millions of ligands investigated. Ligand/protein binding experiments and molecular dynamics calculations are currently ongoing to confirm the docking results and possibly pave the way to the discovery of a new class of ligands with great therapeutic potential.

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FP6

TARGETING TRANSCRIPTION FACTORS IN ACUTE LEUKEMIA

Chaimae El Hadri[a,b], Isaure Dewitte[a] , Filip Matthijssens [c] , Martijn Risseeuw[b] , Pieter Van Vlierberghe [c] Steven Goossens[a], Serge Van Calenbergh [b]

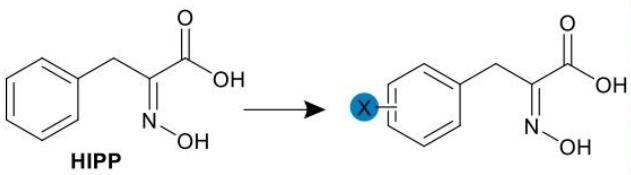
[a] Department Diagnostic Sciences, Ghent University, Ghent Belgium
[b] Laboratory for Medicinal Chemistry, Ghent University, Ghent, Belgium
[c] Department Biomolecular Medicine, Ghent University, Ghent Belgium
Ottergemsesteenweg 460, B-9000 Gent, Belgium

chaimae.elhadri@ugent.be

Acute leukemia is a hematopoietic malignancy characterized by the rapid and aggressive proliferation of immature progenitor cells, typically in the bone marrow. Due to its aggressive nature, acute leukemia is commonly associated with a poor prognosis. The pathophysiology of acute leukemia involves complex genetic and molecular alterations that drive the uncontrolled growth and survival of malignant cells.

Recently, we identified and validated ZEB2 as dependency gene in AML. This transcription factor and its obligate cofactor CtBP1 are involved in the onset and progression of different acute leukemias. Due to the complexity and lack of structural information on ZEB2, we decided to explore the design of PROTACs for CtBP1 and started with the optimization of the known inhibitor 2-hydroxyimino-3-phenylpropanoic acid (HIPP).

We designed, synthesized, and evaluated a library of HIPP derivatives. Utilizing a combination of biophysical assays (nanoDSF), kinetic enzymatic inhibition studies, and isothermal titration calorimetry (ITC), we identified two promising candidates. These lead compounds will be further developed for PROTAC design.



HIPP

	HIPP	CH3020	CH3021
ΔT °C	4.40	**18.76**	**18.33**
IC_{50}(μM)	1.21	**0.33**	**0.28**

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FP7

New Au and Ag NHC complexes with *N*-Boc-protected proline as promising candidates for neurodegenerative diseases

Jessica Ceramella,[a] Domenico Iacopetta,[a] Annaluisa Mariconda,[b] Assunta D'amato,[c] Daniel Chavarria,[d] Francesca Procopio,[e] Francesco Ortuso,[e] Stefano Alcaro,[e] Pasquale Longo,[c] Fernanda Borges,[d] Maria Stefania Sinicropi,[a]

[a] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, via Pietro Bucci, 87036 Arcavacata di Rende, Italy
[b] Department of Science, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
[c] Department of Chemistry and Biology "A. Zambelli", University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy
[d] CIQUP-IMS/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[e] Dipartimento di Scienze della Salute, Campus "S. Venuta", Università degli Studi "Magna Græcia" di Catanzaro, Viale Europa, 88100 Catanzaro, Italy

jessica.ceramella@unical.it

Alzheimer's and Parkinson's diseases are progressive and multifactorial neurodegenerative diseases, affecting mostly older humans [1]. The main players in these pathologies are oxidative stress and chronic inflammation that, affecting each other, alter cerebral levels of neurotransmitters (*e.g.* dopamine and acetylcholine). Indeed, the "switch on" of the inflammatory cascade triggers multiple pathways and responses, including reactive oxygen species (ROS) and cytokines production [2]. In order to restore the normal neurotransmitters levels, different MAO and acetylcholinesterase inhibitors (MAOIs and AChEIs) were developed [3]. Starting from these observations, we synthetized and biologically characterized, through *in silico* and *in vitro* studies, a new series of gold(I) and silver(I) NHC complexes with *N*-Boc-protected proline as promising MAOIs and AChEIs. Their safety, the antioxidant and anti-inflammatory properties have also been evaluated. Our outcomes may contribute to the future advancement of Au and Ag NHC complexes as potential therapeutic multitarget tools for neurodegenerative diseases.

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FP8

Employing virtual screening methods to discover novel BRD4 inhibitors as potential anticancer agents

Federica Chiera,[a] Francesca Alessandra Ambrosio,[a] Giosuè Costa,[a,b] Stefano Alcaro,[a,b,c] Gerhard Wolber [d]

[a] Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100, Catanzaro, Italy

[b] Net4Science S.r.l., Università degli Studi "Magna Græcia" di Catanzaro, 88100 Catanzaro, Italy;

[c] Associazione CRISEA-Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Loc. Condoleo, 88055 Belcastro, Italy;

[d] Department of Pharmaceutical Chemistry, Institute of Pharmacy, Freie University Berlin, Koenigin Luise Strasse, Berlin 14195, Germany

f.chiera@unicz.it

Epigenetics encompasses alterations in gene expression independent of DNA sequence changes. These modifications result from the actions of writers, readers, and erasers. Readers recognize specific epigenetic marks like histone acetylation, affecting transcription and cell signaling. Bromodomain and extraterminal domain 4 protein (BRD4) binds acetylated lysines, implicated in oncogene regulation such as MYC, and holds promise for cancer therapy. In this context, VS techniques have been applied to identify potential BRD4 inhibitors.[1,2] The X-ray crystal structure of BRD4 receptor BD1 (PDB ID: 3MXF) retrieved from the Protein Data Bank, was optimized through the Protein Preparation Wizard *tool*, implemented in Maestro. Virtual Screening was performed using a library of 2498 FDA-approved drugs downloaded from the DrugBank database, prepared using the LigPrep *tool*. Docking studies were performed using Glide. Based on docking score and visual inspection, promising compounds were selected. The best promising complexes were submitted to Molecular Dynamics simulation using Desmond. Dynophore models were generated using the DynophoreApp developed in the Molecular Design Lab at the Freie Universität of Berlin, establishing the critical interactions required for inhibition. These interactions include a direct hydrogen bond with the Asn 140, an indirect hydrogen bond with the Tyr 97 mediated by the structural water molecule, and hydrophobic interactions with residues Trp 81, Pro 82, and Phe 83 of the 'WPF shelf' region. These compounds are currently undergoing biological studies.

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FP9

From a protein crystallography-based fragment screen to the development of novel Sirtuin 6 potential inhibitors.

Emanuele Fabbrizi,[a] Francesco Fiorentino,[a] Clemens Steegborn, [b] Dante Rotili,[a] Antonello Mai.[a]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

[b] Department of Biochemistry, University of Bayreuth, 95445 Bayreuth, Germany

emanuele.fabbrizi@uniroma1.it

As an important NAD^+-dependent enzyme, SIRT6 has received significant attention since its discovery. Recent evidence has demonstrated that SIRT6 functions as a lysine deacetylase, deacylase, and mono-ADP-ribosyltransferase and participates in a variety of cellular signaling pathways including DNA damage repair, metabolic homeostasis, and apoptosis. Furthermore, SIRT6 regulates a wide range of physiological and pathological processes, such as inflammation, cardiovascular diseases (atherosclerosis, cardiac hypertrophy, heart failure, ischemia-reperfusion injury), aging (genomic damage, telomere integrity, DNA repair), metabolism (glycolysis, gluconeogenesis, insulin secretion, and lipid synthesis, lipolysis, thermogenesis), and neurodegeneration [1-2]. SIRT6 regulates the expression and activity of both pro-apoptotic (e.g., Bax) and anti-apoptotic factors (e.g., Bcl-2, survivin) in a context-depending manner. Mounting evidence points towards a double-faced involvement of SIRT6 in tumor onset and progression since the block or induction of apoptosis leads to opposite outcomes in cancer [3]. Given the key roles of SIRT6 in the onset and development of different cancer types, we set out to develop novel SIRT6 inhibitors that may set the ground for new anticancer drugs, while also being useful as chemical tools to further study SIRT6 biology. After screening a fragment library using protein crystallography, we exploited designed several putative hit compounds by merging the best fragments with different linkers. This allowed us to identify novel scaffolds for targeting the SIRT6 pocket, and the result was compound S6023, which showed micromolar activity (IC_{50} = 5 µM). Compound S6023 selectively inhibits SIRT6, and it results active in W138 cells, increasing the levels of the senescence marker p21 and γH2AX. In order to improve the potency of S6023, we developed over thirty analogues. Among these, we identified MC4637, which has a similar binding mode with S6023 according to its co-crystal structure with SIRT6. Notably, in the fluorogenic enzymatic FdL (Fleur-de-Lys) assay, UBCS543 exhibited submicromolar activity (IC_{50} = 600 nM). Finally, MC4637 did not significantly affect the activity of other SIRT isoforms (Sirt1-3 and Sirt5) even at the highest concentration tested (25 µM). Furthermore, it has been observed that at the concentration of 5 µM, MC4637 increased the histone acetylation levels of H3K9 and H3K18 in a dose-dependent manner in HEK T273 cells, highlighting its inhibition of cellular SIRT6 activity.

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FP10

Alternatives to glyphosate: a virtual screening study for the identification of natural inhibitors on Arabidopsis thaliana's EPSP synthase

Marina Bilotta[a], Stefano Alcaro[a,b,c]

[a] Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100, Catanzaro, Italy
[b] Net4Science Academic Spin Off, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100, Catanzaro, Italy Campus "S. Venuta"x5, 88100, Catanzaro, Italy
[c] Associazione CRISEA Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Località Condoleo, 88055 Belcastro, Italy
marina.bilotta@studenti.unicz.it

Glyphosate, a non-selective herbicide widely used in agriculture, is effective but poses potential risks to human health and the environment. It targets the enzyme EPSP synthase (5-enolpyruvylshikimate-3-phosphate synthase) in the shikimate pathway, essential for synthesizing aromatic amino acids like phenylalanine, tyrosine, and tryptophan, crucial for plant growth. Glyphosate inhibits EPSP synthase, blocking this pathway. This study aimed to identify natural bioactive compounds that inhibit EPSP synthase similarly to glyphosate. Using a multidisciplinary approach, alternatives were examined through scientific studies and computational methods. Crystallographic models from the Protein Data Bank, 1G6S [1] and 7PXY [2], were utilized. The 1G6S model pertains to E. coli's EPSP synthase with co-crystallized ligands glyphosate and shikimate-3-phosphate, while 7PXY pertains to Arabidopsis thaliana's EPSP synthase without co-crystallized ligands. Protein structure alignment was performed using Schrödinger's *Maestro*, achieving good alignment score and RMSD value between the PDB models 1G6S and 7PXY. A SiteMap was created to ensure that the resulting binding site aligned with the site identified through UniProt alignment, verifying the protein sequence alignment of the two models. The Arabidopsis thaliana EPSP synthase (PDB: 7PXY) was prepared using the Protein Preparation Wizard, specifying a pH of 7.75, relevant for the chloroplast environment, and using OPLS 2005 as the force field. The docking grid was performed using *Glide* (*Maestro* Schrödinger 2020-04), focusing on the amino acid residues corresponding to those in the bacterial EPSP synthase model (PDB: 1G6S) based on multiple sequence alignment. Glyphosate and shikimate-3-phosphate were docked in 7PXY model to obtain the *cut-off* for subsequent virtual screening studies. The Molport database (natural and purchasable molecules) was used for virtual screening, filtering compounds based on G-score and Lipinski's rules. The most promising hits were identified through visual inspection and clustering using the Canvas software platform with a hierarchical method (MACCS, Tanimoto similarity). Molecular dynamics simulations of 500 ns were conducted with Desmond to evaluate the enzyme's behavior with and without the docked ligands. Subsequently, in vitro studies will be conducted to assess potential enzyme inhibition by selected natural molecules.

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FP11

Development of novel benzamide- based mitochondrial $K_V1.3$ inhibitors with potent anticancer activity

Marzia Fois[a], Špela Gubič[a] ,Luis A. Pardo[b], Boštjan Markelc[c], Maja Čemažar[c], Maša Omerzel[c], Katja Kološa[d], Ivan Džajic[a], Daniela Secci[a], Andrej E. Cotman[a], Lucija Peterlin Mašič[a], Tihomir Tomašič[a]

[a]University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia;
[b]AG Oncophysiology, Max-Planck Institute for Experimental Medicine, Hermann-Rein-Str. 3, 37075 Göttingen, Germany;
[c]Oncology Institute, Department of Experimental Oncology, Zaloška cesta 2, 1000 Ljubljna, Slovenia;
[d] National Institute of Biology, Division of Genetic Toxicology and Cancer Biology, Večna pot 121, 1000 Ljubljana, Slovenia

marzia.fois@ffa.uni-lj.si

$K_V1.3$ is a transmembrane protein, expressed in cellular and in mitochondrial membrane, belonging to voltage-gated potassium channel $K_V1.x$ subfamily. $K_V1.3$ has become an interesting target for anticancer therapy because a correlation between its expression and the development of cancer was demonstrated. It is overexpressed in different types of tumors and its activity is involved in cell proliferation and in the process of apoptosis. [1,2]

The aim of our work is to develop new inhibitors of the mitochondrial $K_V1.3$ (mito$K_V1.3$) channel that would induce the apoptosis of cancer cells. We recently designed, synthesized, and evaluated a new series of benzamide-based mitochondrial $K_V1.3$ inhibitors[3] composed of the thiophene-based $K_V1.3$ inhibitor, a lipophilic alkyl linker and different mitochondria-targeting moieties such as cationic triphenylphosphonium group (TPP^+), substituted triphenylphosphonium group or different pyridinium groups.

The anticancer activity of new compounds was evaluated and compared in different cancer cell models in which a significant toxicity and induction of apoptosis was observed. Moreover, the channel inhibition was investigated by patch-clamp electrophysiology and safety in non-cancer cells. Further biological evaluation is currently in progress.

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FP12

Tyrosinase-related pathologies: identification of novel natural extracts

Alice Fossati[a,b], Valeria Cavalloro[a,b], Simona Collina[c], Emanuela Martino[a,b].
[a] Department of Earth and Environmental Sciences, University of Pavia. Via Ferrata 1, 27100 Pavia, Italy.
[b] National Biodiversity Future Center. Piazza Marina 61, 90133 Palermo, Italy.
[c]Department of Drug Science, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy.

alice.fossati01@universitadipavia.it

Tyrosinase is the key enzyme responsible for the production of melanin in skin cells and neuromelanin in *substantia nigra* dopaminergic neurons[1]. Tyrosinase dysregulation is an underlying mechanism identified in two different pathological conditions: (i) melanoma and (ii) Parkinson's disease (PD). Melanoma is an aggressive skin cancer originating from the malignant transformation of melanocytes. In 2020 approximately 57000 deaths were associated with this tumor worldwide and a 68% increase is predicted by the end of 2040[2]. More than 75% of melanoma cancer cells during tumorigenesis lead to abnormal melanin accumulation and subsequent radiotherapy resistance[3]. Conversely, PD is a progressive, chronic neurodegenerative disease associated with movement disorders. It predominantly affects males over 65 years of age, with an estimated growth to 12.9 million cases by 2040[4]. Although seemingly unrelated, the two pathologies share clinical manifestations (vitamin D deficiency) and risk factors (gender, age, ethnicity)[5]. Overexpression of the enzyme can transform dopamine into toxic oxidizing metabolites and ROS, causative for oxidative stress and neurodegeneration in PD phenotype.
Due to the common key enzyme, evidence of the co-occurrence of the two pathologies has recently emerged[1]. Accordingly, the primary objective of this study is to pinpoint plant extracts that have the potential to relieve symptoms and improve outcomes of tyrosinase-related pathologies. To achieve this, 27 plants belonging to different botanical families were selected via a taxonomic approach. Subsequently, methanolic extracts were prepared, treated to remove chlorophylls and their phytochemical profile drawn. Afterwards, an initial biological screening was performed. More specifically, the inhibitory effects on tyrosinase, acetylcholinesterase, and antioxidant activities have been evaluated. Extracts of *Carissa macrocarpa* (Eckl.) A.DC. and *Adenophora lilifolia* (L.) Ledeb. ex A.DC. proved to be the most promising. Further *in vitro* and *in silico* investigations are ongoing on the two extracts to evaluate their potential to counteract melanoma and PD.

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FP13

Fostering innovation in vector bone parasitic diseases through young researcher innovators within CA21111 *OneHealthDrugs*

Elisa Uliassi,[a] Clara Lima,[b,c] Chiara Borsari,[d] Gülşah Bayraktar,[e] Kayhan Ilbeigi,[f] Maria Laura Bolognesi,[a] Anabela Cordeiro-da-Silva,[b,c] Maria Paola Costi[g]

[a]Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy; [b]Institute for Research and Innovation in Health (i3S), University of Porto, 4200-135 Porto, Portugal; [c]Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, 4050-313 Porto, Portugal; [d]Department of Pharmaceutical Sciences, University of Milan, 20133 Milan, Italy; [e]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, Izmir 35100, Turkey; [f]Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, 2610 Wilrijk, Belgiumg; [g]Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy

elisa.uliassi3@unibo.it

To address vector bone parasitic diseases (VPBDs) affecting both human and animal health, Young Researcher Innovator (YRI) within EU COST-Action CA21111 OneHealthdrugs[1] aims to stimulate research and training activities across various scientific areas in VBPD field, by creating new knowledge and technologies according to a One Health perspective. These activities cover VPBD drug discovery research, One Health, and ecotoxicology aspects and include a wide range of opportunities:

- conference and ITC conference grants, supporting YRI to attend conferences on the OHD topics that are not organized by the COST Action;
- training schools, aimed at training YRI on OHD topics. Next Training School will be on *Cell culture as in vitro models for VBPD drugs,* 25-27 Sept 2024, Poland;
- short-term scientific missions (STSMs), Inter-laboratory Exchange Visits on OHD topics and particularly intended for YRI;
- WG-HG meetings and workshops, check all scheduled OHD events in 2024;
- OneHealth*drugs* marathon organized every November to celebrate the One Health Day with 3-days of non-stop OHD presentations and a session dedicated to YRI;
- OHD surveys investigating different OHD topics;
- Special Issue *One Health and Vector Borne Parasitic Diseases* in ACS Infect. Dis.;
-Women in STEM initiative, a collection of interviews to OHD women scientists;
- OHD newsletter, containing news concerning all OHD activities;
- OHD social media: YOUTUBE channel, LinkedIn and X for OHD updates and video.

By implementing such activities, OneHealth*drugs* will raise awareness and advance innovative and multidisciplinary approaches against VBPD, while minimizing the environmental impact.

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[1] https://www.onehealthdrugs.com

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FP14

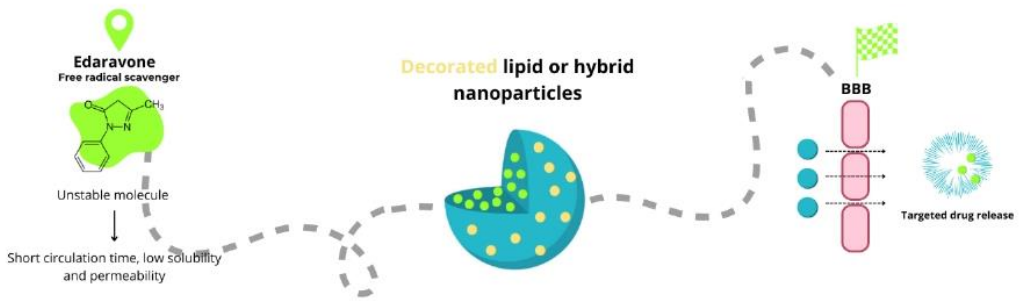
Optimization of Edaravone-loaded lipid and hybrid nanoformulations

Ana Rita Alfenim[a], Brandon Aguiar[a,b,c], Cláudia Machado[a], Fernanda Borges[a] and Carlos Fernandes[a]

[a] CIQUP-IMS – Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, R. Campo Alegre s/n, 4169-007 Porto, Portugal.
[b] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.
[c] UCIBIO – Applied Molecular Biosciences Unit, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.

up201707252@up.pt

Edaravone (EDV) is a free radical scavenger approved by the Food and Drug Administration (FDA) for the treatment of Amyotrophic Lateral Sclerosis (ALS). Nevertheless, it is an unstable molecule with limited clinical application due to its poor water solubility, short half-life, and low permeability[1]. In that sense, nanotechnology-based drug delivery systems arise as a potent tool in terms of inferring drug stability, targeted drug delivery, and longer circulation times, improving the drug's therapeutic effect at the desired site[2]. Considering the capability of nanoparticles (NPs) to cross the blood-brain barrier (BBB), different EDV-loaded lipid and hybrid NPs were prepared by solvent emulsification/evaporation method. Then, it was quantified the amount of loaded EDV, determined the size and surface charge of NPs as well as their storage stability at 4°C. It was predicted the EDV release from NPs at pH 7.4.
This study attempts to create an optimal nanoformulation encapsulating an ALS drug, as treatment options presently consist of only four FDA-authorized active pharmaceutical agents. Specifically, introducing EDV into the brain via a BBB-permeable nanovector may be a potential method for treating ALS with high efficiency and a longer intervention time-window.



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This work was funded by FEDER funds through national funds by FCT – Foundation for Science and Technology under research grants UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS) and 2021.04016.CEECIND/CP1655/CT0004. A.R.A (2023.01250.BD) and B.A. (2020.08731.BD) were supported by FCT. C.F. thanks the FCT for the financial support of his work contract through the Scientific Employment Stimulus — Individual Call (2021.04016.CEECIND/CP1655/CT0004).

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FP15

Design, synthesis, and evaluation of Flavivirus NS3 inhibitors.

Giulia Atzeni[a], Rita Meleddu[a], Alessia Onali[a], Erica Sanna[a], Laura Demuru[a], Simona Distinto[a], Filippo Cottiglia[a], Antonio Lupia[a], Elias Maccioni[a]

[a] Department of Life and Environmental Sciences, University of Cagliari

A Building, Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari, Italy

giulia.atzeni@unica.it

Nowadays, zoonosis caused by Flaviviruses represents a global health concern because they cause serious diseases such as encephalitis for West Nile or haemorrhagic fever for Dengue virus. To date, no antiviral treatments are available [1].

Intending to deal with the high mutation rate and the rapid selection of resistance toward common antiviral drugs, we focused our drug discovery efforts on non-structural protein 3 (NS3), helicase domain, because it is well-conserved (67% sequence identity) across all Flaviviruses [2]. They are positive-sense single-stranded RNA viruses belonging to the *Flaviviridae* family [3]. $NS3^{hel}$ (72k-DA) is essential for viral RNA synthesis; in fact, viruses carrying a defective or impaired $NS3^{hel}$ gene cannot replicate properly. $NS3^{hel}$ requires RNA-stimulated NTPase activity to provide the energy for RNA unwinding and translocation along the dsRNA [4]. With the goal to identify new potential hits, both synthetic and Computer-Aided Drug Discovery (CADD) approaches may prove worthwhile in developing new $NS3^{hel}$ inhibitors for Flavivirus (Figure 1).

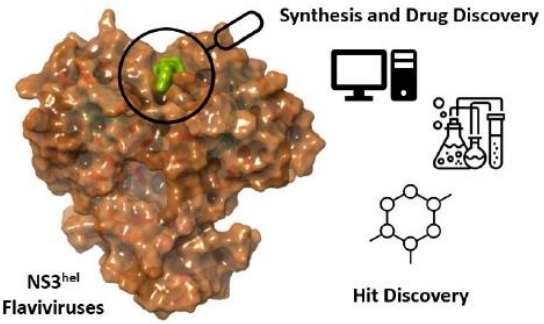

Figure 1. Synthetic and CADD approaches to identify new antiviral agents.

"This research was supported by EU funding within the Next GenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases".

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FP16

Application of Computational Methods for the identification of NS3^{pro} ZIKV potential allosteric inhibitors

Laura Demuru[a], Antonio Lupia[a], Erica Sanna[a], Alessia Onali[a], Giulia Atzeni[a], Filippo Cottiglia[a], Simona Distinto[a], Elias Maccioni[a], Rita Meleddu[a]

[a] Affiliation Department of Life and Environmental Sciences, University of Cagliari

Affiliation Address A Building, Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari, Italy

laura.demuru2@unica.it

Zika Virus (ZIKV) belongs to the Flavivirus genus, and the infections it causes, transmitted by Aedes species mosquitoes, pose an ongoing threat to public health. The positive-sense single-stranded RNA genome of *Flaviviridae* family members encodes for a single polyprotein co- and post-translationally cleaved by viral and cellular proteases, into structural and non-structural (NS) proteins.[1] Among the NS proteins, the highly conserved NS2B-NS3 protease, characterised by the catalytic triad – S135, H51, D75 – at the N^{ter} region, is essential for the cleavage and the viral replication, representing a valid druggable target[2]. The structural homology between the active centre of NS3^{pro} and various host serine proteases, as well as the inefficacy to date of peptide derivatives covalent inhibitors, make their development challenging. Thus, the identification of NS3^{pro} allosteric inhibitors appears as an alternative strategy, also considering that both vaccines and drugs are not commercially available. In our approach, *in silico* molecular dynamics, dynophore, virtual-screening, and synthetic methods, were applied with the aim to identify new potential hits.

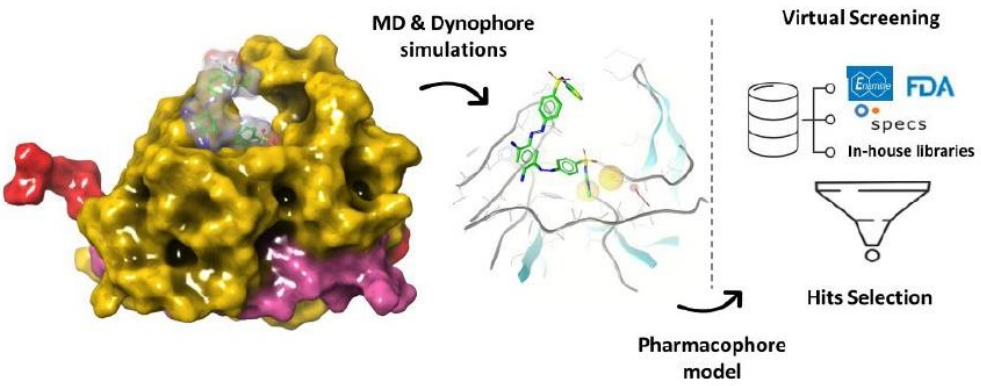

Figure 1. In silico workflow.

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FP17

De-ironing Friedreich's Ataxia with innovative mitochondria-targeted chelators

Carla Lima,[a] Catarina Alves[a], Daniel Chavarria[a], Lisa Sequeira[a], Fernando Cagide[a],

Fernanda Borges[a], Sofia Benfeito[a]

[a] CIQUP-IMS- Centro de Investigação em Química, Instituto Ciências Moleculares Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal;

Rua do Campo Alegre 1021 1055, 41689-007, Porto, Portugal

up201705563@up.pt

Friedreich's Ataxia (FRDA; ORPHA:95) is an inherited and progressive neurodegenerative disease, being characterized by sensory loss and hypertrophic cardiomyopathy. The FRDA is the most common form of inherited ataxia with an autosomal-recessive inheritance pattern, affecting 1 in 50.000 individuals worldwide, that is typically diagnosed in childhood (2-3 years) or early adulthood (>25 years) and often progresses into a fatal outcome.

FRDA is related to decreased levels of the frataxin (FXN), a mitochondrial protein that plays a key role as iron chaperone in the synthesis of heme and iron-sulfur clusters (ISCs). The FXN decreased levels are caused by homozygous hyperexpansion of guanine-adenine-adenine (GAA) triplets in the first intron of the FXN gene on chromosome 9. Sustained reduction of FXN amount leads to increased levels of mitochondrial labile iron, impairments in ISC biogenesis, reduction in heme biosynthesis and defective activities of aconitase and respiratory chain complexes I, II and III. These events result in ROS overproduction, reduced ATP production and, ultimately, in mitochondrial dysfunction. Some of the molecular mechanisms of FRDA also trigger oxytosis/ferroptosis, a form of iron-induced cell death. Despite massive efforts, there is no current approved drug to prevent/delay the progression of FRDA.

Deferiprone (DFP) is an iron chelator used in treatment of thalassemia and, due to its low molecular weight, favorable hydrophobicity and neutral charge, it becomes a privileged structure in drug discovery programs. In this way, a new library of compounds based on DFP was synthesized aiming to act on oxidative stress, maintaining the iron chelating capacity of its precursor. Structural characterization of the newly synthesized compounds was carried out by NMR (1H, ^{13}C and DEPT) spectroscopy. The synthesis obtained so far will be presented in this communication.

Acknowledgements: This work was funded by FEDER funds through the Operational Program Competitiveness Factors COMPETE and national funds by the FCT-Foundation for Science and Technology under research grants [PT-OPENSCREEN-NORTE-01-0145-FEDER-085468, LA/P/0056/2020, UIDB/00081/2020, UIDP/00081/2020 and 2022.04633.PTDC-DEFEND project. CL, DC, LS, FC and SB thanks FCT, POPH, FEDER/COMPETE for their contracts/grants (CL UI/BD/154557/2023; FC: 10.54499/DL57/2016/CP1454/CT0001 and SB: 2023.06106.CEECIND).

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FP18

Preclinical investigation of H80 compared to miltefosine: imaging and proteomics for sustainable drug development.

Giulia Malpezzi[a], Lorenzo Tagliazucchi[a], Daniele Aiello[a], Alberto Venturelli[a], Anabela Cordeiro-da-Silva[b], Nuno Santarem[b], Glauco Ponterini[a], Maria Paola Costi[a]

(a) Dept. of Life Sciences, University of Modena and Reggio Emilia, via Campi 103, 41126, Modena, Italy. (b) Instituto de Investigação e Inovação em Saúde and IBMC-Institute for Molecular and Cell Biology, Universidade do Porto and Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto (FFUP), 4150-180 Porto, Portugal;

giulia.malpezzi@unimore.it

Leishmaniasis treatment demands safer, orally available drugs with shorter treatment durations than current options. Miltefosine (MIL), while effective, comes with severe side effects. Our research aims to surpass MIL's limitations by discovering compounds like H80, which has shown potent activity against various Leishmania strains with minimal drug resistance. Our study targets understanding H80's mechanism of action and molecular targets, crucial for rational drug design. Using fluorescence imaging and mass spectrometry-based proteomics, we analysed protein expression profiles of Leishmania parasites treated with H80 and MIL. Our findings revealed significant overlap in differentially expressed proteins (DEPs) between H80 and MIL treatment, particularly those involved in membrane transport and biosynthesis. This convergence suggests shared pathways impacted by both compounds, offering insights into their mechanism of action.

Furthermore, fluorescence imaging unveiled H80's cellular uptake mechanism, indicating endocytosis-mediated internalization and cytoplasmic localization within parasites. These observations were reinforced by immunofluorescence assays. Moving forward, our research will delve deeper into the biochemical pathways modulated by H80 compared to MIL. Leveraging proteomic techniques, such as LC-MS/MS analysis on amastigotes, we aim to delineate specific protein interactions influenced by H80. In conclusion, our study sheds light on H80's therapeutic potential in Leishmaniasis treatment. By unravelling its mechanism and cellular dynamics, we pave the way for rational drug design strategies and the development of safer, more effective treatments for this disease.

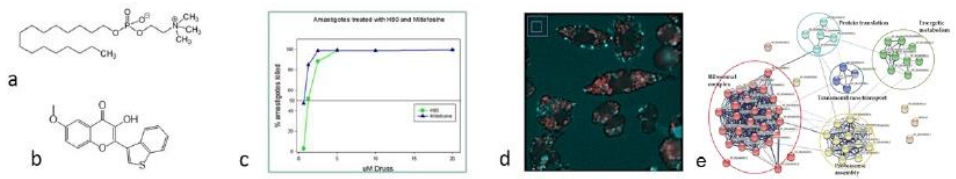

Figure 1. (a) Miltefosine structure. (b) H80 structure. (c) EC50 of Miltefosine and H80 in L *infantum* amastigotes. (d) fluorescence-based immunoassay for internalization study of H80 (e) MS samples were analysed with Progenesis (Waters) with a label free approach and the main pathways were studied with String software (STRING Consortium 2023)

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FP19

Thiosemicarbazone as inhibitors of metallo-β-lactamase NDM-1

Msanif Msanif[1], *Michał Jewgiński*[1], *Rafał Latajka*[1]
[1]*Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław*
276784@student.pwr.edu.pl

Resistance of Gram-negative bacteria to β-lactam antibiotics, including carbapenems, stems from the activity of a group of β-lactamase enzymes that effectively hydrolyze and inactivate these compounds. Increasing treatment options for multidrug-resistant infections requires the development of β-lactamase inhibitors (BLIs) to restore the effectiveness of β-lactam antibiotics [1]. Considering the increasing bacterial resistance to β-lactam antibiotics, we focus on the urgent need to develop and optimize metallo-β-lactamase (MβL) class inhibitors offering enhanced selectivity and efficacy. MβLs can hydrolyze almost any β-lactam, including carbapenems. The New Delhi Metallo-β-Lactamase (*NDM-1*), which belongs to this class, has been identified mainly in *Escherichia coli*, *Klebsiella pneumoniae,* and to a lesser extent in *Pseudomonas* and *Acinetobacter*, resulting in especially wide antibiotic resistance. The high ability of this enzyme to spread promptly, derived from the horizontal plasmid-encoded gene (bla_{NDM-1}) transfer, carries the additional risk of drug resistance spreading. In addition, the deep cavity of the NDM-1 active center, including two flexible domains and two Zn^{2+} ions, provides specificity and selectivity, making the active site suitable for accommodating a wide range of β-lactams that many other MβLs are unable to hydrolyze [2,3].

Our research focuses on the design of novel metal-β-lactamase (MβL) inhibitors, especially NDM-1 based on the thiosemicarbazone scaffold (Figure 1). Optimization of the inhibitors' structures is based on structure-activity relation studies to achieve IC50 values below 1μM for at least one inhibitor. Since thiosemicarbazones and thiosemicarbazides possess activity against some metal-β-lactamases, we focus on the modification of R1, R2 R3, R4 substrates based on compounds, including aromatic rings, heterocyclic analogs with thiophene or pyrrole ring, as well as ebselen-derived rings.

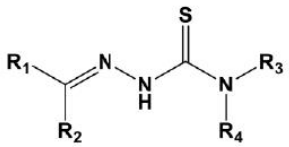

Fig.1 The thiosemicarbazone scaffold used for inhibitor design.

The goal is to develop inhibitors for NDM-1 that can bind zinc ions crucial for its catalytic activity and also form covalent bonds with cysteine residues in its active site. This involves using techniques like molecular modeling and docking simulations. These inhibitors could offer new ways to fight drug-resistant bacterial infections.

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FP20

Deciphering host-parasite interplay in *Leishmania* infection through a One Health view of proteomics studies on drug-resistance

Lorenzo Tagliazucchi [a], Giulia Malpezzi [a], Ana Perea Martinez [b], Alexander R. Cole [c], Daniele Aiello [a], Ba Reum Kwon [c], Bryan W. Brooks[c], Eli S. J. Thoré [d], Michael G. Bertram [d], Francisco Gamarro [b], Maria Paola Costi [a]

[a] Department of Life Sciences, UniMoRe, Via G. Campi 103, 41125 Modena, Italy;

[b] Institute of Parasitology and Biomedicine ''López-Neyra'', Avda. del Conocimiento 17, 18016- Armilla (Granada), Spain.

[c] Environmental Health Science Program, Department of Environmental Science, Baylor University, One Bear Place 97344, Waco, Texas, USA.

[d] Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Skogsmarksgränd 17, 907 36 Umeå, Sweden.

lorenzo.tagliazucchi@unimore.it

There is an urgent need to develop new pharmaceuticals to overcome drug resistance issue in Leishmaniases, as the commonly used antimonials, paromomycin, and miltefosine show very limited efficacy due to the emergence of hyper-resistant parasitic strains. Therefore, we combined the performances of omics sciences, including MS proteomics and RNA-seq transcriptomics to investigate the parasitic biochemical modifications associated with drug resistance patterns. By exploiting Bioinformatics tools, we have investigated the role of the 14 most significant proteins from the previous analysis in the guest-host crosstalk between parasites and the human monocytes. Finally, through a phylogenomic-driven Ecotoxicological investigation of the hit proteins, we evidenced a particular Calpain-like peptidase to represent a putative ideal drug target to start a new Medicinal Chemistry Programme.

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FP21

Acyclic nucleosides as potential antikinetoplastid agents

Julie Traen[a], Serge Van Calenbergh[a], Guy Caljon[b]

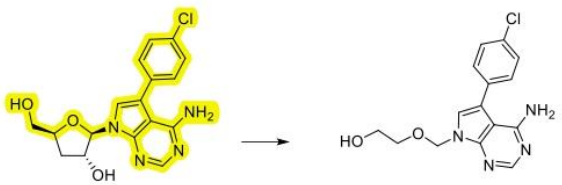
[a]Laboratory of Medicinal Chemistry, Ghent University, Ghent, Belgium
[b]Laboratory of Microbiology, Parasitology and Hygiene, Antwerp University, Antwerp, Belgium

Ottergemsesteenweg 460, 9000 Gent, Belgium
Universiteitsbaan 1, 2610 Wilrijk, Belgium

Julie.traen@ugent.be

Neglected tropical diseases (NTDs) encompass various infections caused by microorganisms like bacteria (e.g., Buruli ulcer), fungi (e.g., chromoblastomycosis), viruses (e.g., rabies), and protozoa (e.g. Human African trypanosomiasis, Chagas disease, leishmaniasis). These diseases are labelled "neglected" because they historically received minimal attention from pharmaceutical companies, despite their significant impact on public health and economic development.[1] Around 200,000 people die from NTDs annually, and over 1.7 billion require treatment each year. Existing therapies suffer from limited efficacy, resistance development, side effects leading to treatment discontinuation and non-oral administration etc. [2]

The therapeutic utility of nucleoside analogues has been well established for viral infections and in the oncology field, but it has been poorly explored for protozoan diseases.[3] This has inspired our research group to synthesize an in-house nucleoside analogue library and evaluate it primarily against a representative panel of *Trypanosoma* and *Leishmania*. Their selectivity is assessed by determining toxicity against two relevant mammalian cell lines. This led to the discovery of several successful 7-deazanucleosides such as **1** and **2**. However the exact mode of action of these compounds is not elucidated yet.[4,5] This has motivated me to investigate the SAR of these compounds further by synthesizing their acyclic version. Acyclic nucleosides are successful antiviral drugs with as example acyclovir against HSV. However, their antiprotozoal activity remains underexplored which makes it interesting to explore this acyclic ribose scaffold combined with the 7-deazapurine against a panel of protozoa.[6]



1
T. cruzi
EC_{50} = 0.047 ± 0.006 µM
J. Med. Chem. ***2018***

2
T. brucei
EC_{50} = 0.048 ± 0.009 µM
Nat. Commun. ***2019***

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FP22

Nature Aided Drug Discovery: preparation and biological evaluation of extracts from pruning wood of *Vitis sp*

Elisabetta Tumminelli,[a] Daniela Ratto [b], Valeria Cavalloro [c], Emanuela Martino [c], Chiara Ingrà [d], Alessandra Ferrandino [d], Simona Collina [a], Paola Rossi [b], Daniela Rossi [a]

[a]Department of Drug Sciences (University of Pavia, Viale Taramelli 12, 27100 Pavia,Italy);
[b] Department of Biology and Biotechnology (University of Pavia Via A. Ferrata, 9, 27100 Pavia PV)
[c]Department of Earth and Environmental Sciences, (University of Pavia Via S. Epifanio, 14, 27100 Pavia, Italy)
[d] Department of Agricultural, Forestry and Food Sciences (DISAFA) (University of Torino Largo P. Braccini, 2

elisabetta.tumminelli01@universitadipavia.it

The pruning process of vines annually generates 2 to 5 tons of wood waste[1].
With a view to recycling, the main focus of this work was to valorize this waste by preparing extracts using different organic solvents under mechanical stirring (dynamic maceration) and evaluating their anticancer potential. To ensure accurate results, a method for removing interferences was also devised comparing activated charcoal and polyvinylpolypyrrolidone (PVPP) [2] for their effectiveness in removing interferences such as chlorophylls or tannins, which can cause false positives in cytotoxicity tests.
Briefly, the extraction of pruning wood of *Vitis vinifera* and *Vitis amurensis* (collected from 16 years old vines grown at the experimental vineyard of DISAFA – Unito, Grugliasco) was performed through dynamic maceration using organic solvents with increasing polarities (n-hexane, dichloromethane, ethyl acetate and a mixture of ethanol/water 50/50, v/v). Extracts, after the application of the optimized interference removal method, were investigated for their cytotoxic properties (MMT and trypan blue assays) in tumoral cell lines (glioblastoma and breast cancer) and normal fibroblast. Results will be presented and discussed in due course. This contribution is part of the project NODES which has received funding from the MUR – M4C2 1.5 of PNRR funded by the European Union – Next Generation EU (Grant agreement no. ECS00000036)

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FP23

Design, Synthesis and Biological Testing of Novel Compounds Containing Sulfonamide and Thiazolidinone moiety as Potential Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase

Maria Fesatidou[1*], Kleio Giortsiou[1], AnthiPetrou[1], AthinaGeronikaki[1]
[1] Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki,Thessaloniki 54124, Greece
*fesa.maria@gmail.com

Human immunodeficiency virus type I (HIV-1) is a retrovirus that infects cells of the host's immune system, which leads to Acquired Immunodeficiency Syndrome (AIDS) and potentially to death. HIV-1 remains a major health problem worldwide, although many drugs are used to prevent its progression. Simultaneously, the virus has developed drug-resistant strains, so, the need for novel agents that can inhibit HIV-1 replication is urgent. The protein reverse transcriptase (RT) of the virus plays fundamental role in the viruses' replication cycle and many of the approved medications aim this target [1]. Thiazolidinones and sulfonamides are two very important scaffolds in developing new HIV-1 reverse transcriptase inhibitors [2-3].
Based on this, herein we present the synthesis of thirty new derivatives (Figure 1.) and the results of their biological activity. For the evaluation of the HIV-1 Reverse Transcriptase activity, the colorimetric photometric immunoassay kit provided by Roche was used.Nevirapine was used as reference drug. Eight compounds exhibited very good activity, while two of them showed better activity than the reference drug.

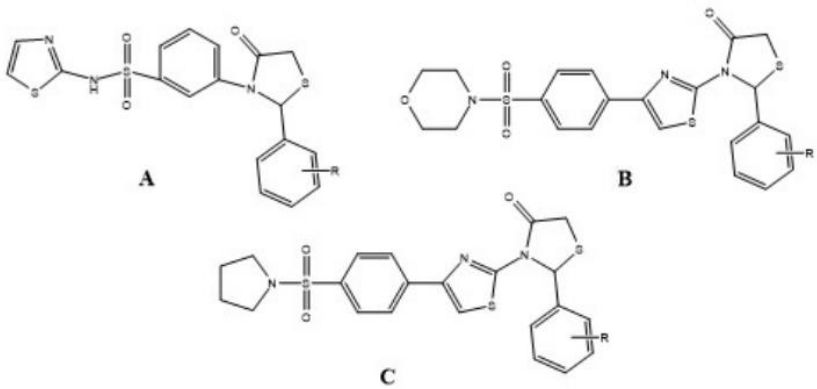

Figure 1. General Structures of the synthesized compounds, Series A, B and C.

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FP24

Development of a second generation of ArnT inhibitors: Abietane-type diterpenoids

Arianna Speroni[1], Silvia Cammarone[1], Mattia Mori[2], Deborah Quaglio[1], Francesca Ghirga[1], Mariya Ryzhuk[1], Bruno Botta[1], Francesco Imperi[3], Fiorentina Ascenzioni[4]

[1]Department of Chemistry and Technologies of Drug, Sapienza University of Rome, 00185 Rome, Italy;

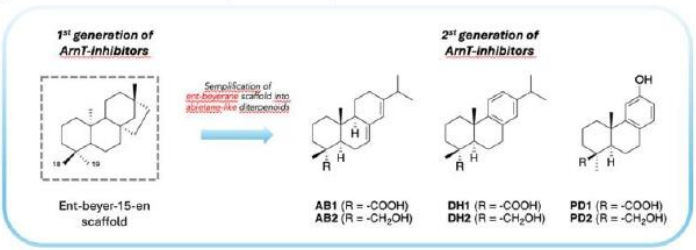
[2]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy;

[3]Department of Science, Roma Tre University, 00146 Rome, Italy

[4]Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, 00185 Rome, Italy.

E-mail: arianna.speroni@uniroma1.it

Colistin is a last-line antibiotic used for the treatment of multidrug resistant Gram-negative bacterial infections. However, a resistance phenomenon due to the glycosyltransferase enzyme ArnT has been recently documented[1]. By combining microbiological assays and molecular modeling, we have previously demonstrated that the diterpene scaffold is a promising platform for the development of novel ArnT inhibitors[2]. In order to further optimize this scaffold, we set up a rational procedure that simplifies the ent-beyerane scaffold into drug-like synthetic molecules. We have been able to select the abietic, dehydroabietic and podocarpic acid (AB1, DH1, PD1) as profitable starting points for the development and synthesis of a second generation of ArnT inhibitors. Furthermore, with the aim of enlarging the abietane derivatives library, the corresponding hydroxylated derivatives (AB2, DH2, PD2) were synthesized, tested against colistin-resistant *Pseudomonas aeruginosa* strain and their binding mode was investigated through molecular docking simulations. These studies highlighted PD2 as the most promising compound to restore colistin sensitivity in bacteria. Thus, our efforts are focused on the synthesis of new derivatives of PD2 with the aim to investigate the SAR of the podocarpic scaffold.



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FP25

Identification of novel Quorum Sensing Interfering Agents against *S. aureus* and *P. aeruginosa*

Giorgio Milli[a], Angelica Pellegrini[b], Roberta Listro[a], Giacomo Rossino[a]Giampiero Pietrocola[b], Pasquale Linciano[a], Simona Collina[a]

[a]Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy); [b]Department of Molecular Medicine, Biochemistry Unit University of Pavia, Viale Taramelli 3/b, 27100 Pavia, Italy
roberta.listro@unipv.it

Antimicrobial resistance (AMR) and superbug infections were recognized from the World Health Organization (WHO) as the global obstacle for public health and the sustainability of healthcare systems[1]. To counteract the insurgence of AMR, the development of new pharmaceutical tools based on the research of novel targets and innovative approaches have been exploited. Among them, focusing on Quorum Sensing (QS) represents a winning strategy in the AMR field, thus identifying novel compounds able to inhibit the bacteria communication event. Specifically, LsrK enzyme represents an innovative target involved in QS. Indeed, after the phosphorylation mediates by LsrK kinase (in both Gram-positive and Gram-negative bacteria) of small internal molecules known as autoinducers, such as 4,5-Dihydroxy-2,3-pentandione (DPDP), the QS has been triggered[2].

Since the LsrK relevant role in QS and consequently in Biofilm formation, this work is focused on the identification of novel DPDP derivatives targeting biofilm-associated infections as QS inhibitors.

We synthesised a library of 21 compounds evaluating their ability to inhibit biofilm formation caused by *P. aeruginosa* and *S. aureus*. The most effective agents, with IC50 value of low micromolar range, were subjected to detailed biological investigation. Since bactericidal and bacteriostatic effects weren't shown, a specific activity of the compounds was highlighted. Moreover, different spectroscopic techniques, including differential scanning fluorimetry (DSF), intrinsic tryptophan fluorescence (ITF), circular dichroism (CD), and nuclear magnetic resonance (NMR) were performed to confirm the mechanism of action.

Our study allows to identify novel potential QS inhibitors targeting LsrK pathways to prevent the biofilm formation in several infections.

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FP26

Acaricidal activity of essential oil and crude extracts of *Laurus nobilis,* (Lauraceae), against *vector of several major pathogenic diseases* in livestock and poultry in Tunisia.

Essia Sebai[2], Amel Abidi[2] and AKKARI Hafidh[1]

[1]Laboratory of Parasitology, National Veterinary School of Sidithabet, Tunisia
[2]Laboratory of Bioactive Substances, Centre of Biotechnology of Borj Cedria, Box 901, Hammam-Lif, 2050, Tunisia

*E-mail adress: akkari_hafidh@yahoo.fr (WG3)

ABSTRACT

The current study assayed the toxicity of *Laurus nobilis* essential oil and crude extracts obtained using solvents of increasing polarity (cyclohexane, acetone and ethanol), on two ectoparasites of veterinary importance, i.e., *Hyalomma scupense* and *Dermanyssus gallinae*.
The major components detected in bay laurel essential oil were dominated by 1.8-cineole (46.56%), a-terpinenyl acetate (13.99%), sabinene (7.69), a-pinene (5.75), linalool (5.50), methyleugenol (5.36%) and -pinene (3.97). The highest total phenolic and flavonoids contents were present in the ethalonic extract of *L.nobilis* leaves at an amount of 152.88 mg gallic acid equivalents per gram of dry weight (GAE/g DW) and 21.77 mg quercetin equivalent per gram of dry weight (QE/g DW) , respectively.
In vitro acaricidal effects of essensial oil and crude extract of *L.nobilis* against *H. scupense* were ascertained by adult immersion test of engorged females (AIT) and larval packet test (LPT) compared with a reference drug amitraz. The essential oil exhibited strong acaricidal activity against tick engorged female and inhibition of hatching eggs. After 24h of exposure, at the highest tested concentration (100 mg/ml), essential oil induced 90.67% mortality of *H. scupense* larvae (LCso = 10.69 mg/mL). Otherwise, essential oil exhibited high acaricidal activity compared to extracts, and among the extract, the ethanolic extract revealed the highest acaricidal efficacy (81.27% female mortality).
Results from mite contact toxicity showed that essential oil and extracts from *L.nobilis* were toxic to *D. gallinae.* Bay essential oil was both more

toxic to mites, and faster in exerting this toxicity than other tested crude extracts. *L.nobilis* essential oil concentration leaded to enhance mortality of *D. gallinae* reaching the highest (100%) mortality at 12h with a concentration of 320 mg/ml. While, ethanolic extract acheived this rate after 24h of exposure at same concentration. Cyclohexanic extract showed weak acaricidal activity.

Acknowledgements.

This work received financial support from Laboratory of Bioactive Substances, Centre of Biotechnology of Borj Cedria, Tunisia.

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FP27

New Morpholino-Uridine and-Adenosine Derivatives as Potential Anticancer Agents

Rasha Ghanem Kattoub,[a,b,c] Miklós Bege,[a,d,e] Erika Hevesi-Mező,[a] Agnieszka Olejarz-Maciej,[b] Tadeusz Karcz,[b] Jadwiga Handzlik,[b] Anikó Borbás[a,d]

[a] Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary.
[b] Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, 30-688 Kraków, Poland.
[c] Doctoral School of Pharmaceutical Sciences, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary.
[d] HUN-REN-DE Molecular Recognition and Interaction Research Group, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary.
[e] Institute of Healthcare Industry, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary.

rasha.ghanemkattoub@pharm.unideb.hu

Abstract

Over the last decade, morpholino-nucleoside-based therapeutics have proven to be highly effective in treating a wide range of diseases. While these structures have shown huge success in the field of antisense-based treatment,[1] much less effort has been employed to explore the potential biological activities of substituted morpholino-nucleoside monomers. On the other hand, synthetic conformationally constrained nucleosides and numerous natural analogues bearing a bi- or tricyclic carbohydrate unit instead of the natural furanose ring have been shown to possess a wide range of biological activities.[2] To further explore the biological properties of morpholino-nucleosides, herein we report the synthesis of a new type of *N*-substituted monocyclic and novel fused bicyclic morpholino-adenosine and uridine (**Fig. 1**).

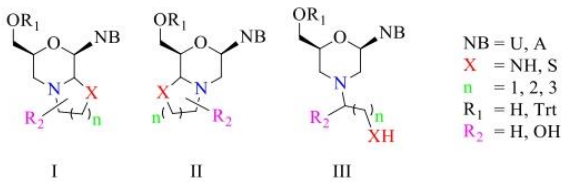

***Fig.1** General structures of synthesized compounds (**1-12**)*

The key reaction used is the double reductive amination (DNR) cyclization of 2',3'-secodialdehydes, prepared by the oxidative cleavage of the corresponding ribonucleosides, with various amines. Out of the applied conditions, two major routes were proven to be effective- the $ZnCl_2$-mediated method and the glacial acetic acid strategy- affording a diverse range of 3'- and 5'-bicyclic morpholino nucleosides, as well as open-chain products. All newly synthesized analogues were evaluated for anticancer activity against PC-3 prostate cancer cell lines. Among all compounds tested, 11 bicyclic and open-chain products showed low to moderate anticancer action, while the open-chain morpholino-adenosine derivative, RK-31-2 (**12**), showed significant growth inhibition with an IC_{50} value of 2.61 µM. Comparing the inhibitory properties of this derivative with the closed form and with its uridine counterpart reveals that the effect depends on both the presence of the primary amine group and the type of nucleobase. This work further validates the importance of morpholino-nucleoside monomers with wide applications in medicinal chemistry.

This project is supported by the National Research and Development and Innovation Office of Hungary (OTKA K 132870), the University of Debrecen Scientific Research Bridging Fund (DETKA), and Jagiellonian University project no. N42/DBS/000331.

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FP28

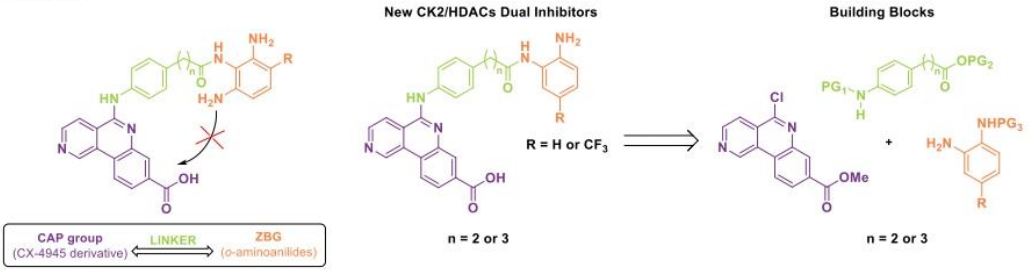
The challenge of achieving an orthogonal pathway in the synthesis of a new generation of CK2/HDACs dual inhibitors

Alba Gil-Rivas, Irene Ortín, Beatriz de Pascual-Teresa and Ana Ramos

Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28668 Boadilla del Monte, Spain

alba.gilrivas@usp.ceu.es

The effectiveness of single-target drug or combination therapies for cancer treatment has been hindered by problems such as resistance, low efficacy, toxicity, dose limitations, and poor patient compliance. To overcome these boundaries, dual inhibitors have been developed, combining two pharmacophores in a single entity to efficiently and simultaneously inhibit two proteins overexpressed in cancer. This approach results in synergistic effects and improved pharmacokinetic properties.[1] Our research focuses on dual inhibitors targeting Protein Kinase (CK2) and Histone DeACetylases (HDACs), both of which are involved in cancer and are interrelated under hypoxia conditions due to CK2´s crucial role in cell growth processes that activates HDACs.[2,3]



In a previous Structure-Activity Relationship study of a new series of CK2/HDACs dual inhibitors featuring *o*-aminoanilides as zinc-binding groups, we explored different linker types (e.g. aliphatic, aromatic ring or triazole) and lengths. The IC_{50} results against both enzymes indicated that the linker plays a pivotal role in the inhibitory activity. However, cell assays revealed a significant loss of activity, which we hypothesise is due to an intramolecular lactamization under acidic conditions, resulting in the loss of the two pharmacophores. To address this challenge, we designed a new synthesis incorporating an aryl group or an electron-withdrawing group ($-CF_3$) to either rigidify the linker or decrease the nucleophilicity of the aniline, respectively, thereby preventing cyclization. The challenge lies in the right use of protecting groups to achieve the desired compounds, which are currently undergoing biological evaluation.

Financial support from PID2021-123786OB-I00 (MICIU/FEDER, UE) is kindly acknowledged. A.G. is supported by a predoctoral research fellowship granted by CEU-Santander.

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FP29

Novel Nortopsentin-Derived 1,3,4-Oxadiazole and 1,3,4-Thiadiazole Compounds: A Promising Leap Towards Effective Pancreatic Cancer Therapy

Silvia Gulino[a], Daniela Carbone[a], Camilla Pecoraro[a], Stella Maria Cascioferro[a], Barbara Parrino[a], Elisa Giovannetti[b], Patrizia Diana[a]

[a] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 32, 90123 Palermo, Italy

[b] Department of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, VU University Medical Center (VUmc), De Boelelaan 1117, 1081 Amsterdam, Netherlands

silvia.gulino@unipa.it

Pancreatic ductal adenocarcinoma (PDAC) poses a significant challenge in oncology due to its aggressiveness, late diagnosis, and limited treatment options. [1-2]

This study addresses the urgent need for novel therapeutic strategies by focusing on the synthesis and biological evaluation of a series of Nortopsentin-derived compounds featuring 1,3,4-oxadiazole and 1,3,4-thiadiazole motifs for potential PDAC treatment.

A library of novel compounds was synthesized via a multi-step chemical approach, strategically incorporating the Nortopsentin scaffold known for its antitumor properties with 1,3,4-oxadiazole and 1,3,4-thiadiazole functionalities to enhance biological activity. Cytotoxicity assessments against a panel of PDAC cell lines using SRB assays revealed several compounds with significant cytotoxic effects, demonstrating potent activity with IC_{50} values in the micro-submicromolar range. Notably, derivatives containing the 1,3,4-thiadiazole moiety exhibited promising results, reaching or surpassing existing treatments in efficacy in certain instances.

Mechanistic investigations uncovered that the active compounds act through the inhibition of cyclin-dependent kinase 1 (CDK1), a pivotal enzyme in cell cycle regulation. Inhibition of CDK1 led to apoptosis induction, as evidenced by increased caspase-3 activation and G2/M phase cell cycle arrest.

These findings underscore the therapeutic potential of 1,3,4-oxadiazole and 1,3,4-thiadiazole Nortopsentin derivatives against PDAC. Targeting CDK1 presents a novel avenue for combatting this untreatable disease. Further preclinical studies are warranted to optimize these compounds for clinical translation, focusing on enhancing efficacy and safety profiles.

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FP30

New 20-Aminopregnenolone Derivatives as Potential Dual-site Binding Acetylcholinesterase (AChE) Inhibitors

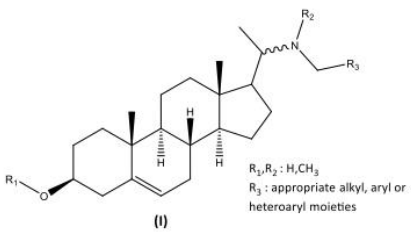
Maria Papadrakaki*, Aristea Tsante*, Georgia Michalopoulou*, Paraskevi Sinou**, Fotini Lamari**, Sotiris Nikolaropoulos*, Manolis Fousteris*

*Laboratory of Medicinal Chemistry, Department of Pharmacy, University of Patras, Patras GR-26500, Greece; E-mail: manolisf@upatras.gr

**Laboratory of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy, University of Patras, Patras GR-26500, Greece

The steroidal skeleton has been recognized as a prominent scaffold for drug discovery.[1] Pregnenolone, an endogenous neurosteroid, exhibits neuroprotective and anti-inflammatory properties.[2] Pregnane-type aminosteroids of synthetic and natural origin have shown promising *in vitro* and *in vivo* acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition.[3] The cholinesterases are serine hydrolases responsible for the breakdown of acetylcholine (ACh), the main neurotransmitter of the cholinergic system. The reduction of ACh in the brain and subsequent cholinergic atrophy are connected to cognitive decline, characteristic of neurodegenerative diseases like Alzheimer's Disease (AD).[4] The multifactorial pathogenesis of AD prompted the development of multi-target directed ligands (MTDLs), which, due to their synergistic activity, enable simultaneous modulation of multiple molecules (e.g. amyloid beta, monoaminoxidase, beta-secretase, tau protein) implicated in AD pathogenesis pathways.[5]

The present study focuses on the discovery of new 20-aminopregnenolone derivatives (**I**) as potential dual-site binding AChE inhibitors, with prospective MTDLs' profile. By applying a structure-based design approach, a library of 20-amino-substituted pregnenolone derivatives, potential binders on the AChE active site, was designed. The designed compounds, which incorporated vital structural motifs for potent AChE inhibition, were introduced to an X-ray crystal structure of human AChE through molecular docking simulations using OEDocking 4.1.1.0.[6] Derivatives which exhibited high binding energies and crucial interactions to the enzyme's active site were further docked to X-ray crystal structures of human monoaminoxidase B (MAO-B) and human beta-secretase 1 (BACE1). Selected compounds with the most favorable binding profile were chosen to be synthesized. The newly synthesized derivatives will be tested *in vitro* to evaluate their AChE, MAO-B, and BACE1 inhibitory activity. Herein, the results of our computational studies, synthetic efforts towards the target compounds and their preliminary biological evaluation regarding their anti-AChE activity will be presented.



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FP31

Monitoring cellular metabolism using NMR and LC-MS spectroscopy, and analyzing changes in cellular metabolome during oxidative stress

Monika Sapeta[a], Katarzyna Gębczak[b], Piotr Młynarz[a]

a Department of Biochemistry, Molecular Biology and Biotechnology, Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland

b Department of Basic Medical Science, Wroclaw Medical University Borowska 211, 50-556 Wroclaw, Poland

monika.sapeta@pwr.edu.pl

Chronic inflammation is a consequence of long-term acute inflammation, potentially contributing to the development of various chronic diseases, including obesity, neurodegenerative disorders, metabolic syndromes, and cancer. One potential cause of inflammation is the accumulation of reactive oxygen species (ROS) [1]. They may be generated in situ or introduced from the external environment. Due to the body's exposure to environmental and pathological factors such as atmospheric pollution, toxic chemicals, pesticides, and the consumption of processed food, the imbalance between the number of free radicals (ROS) and antioxidants generated may occur, which leads to oxidative stress. When ROS levels increase, they begin to have a detrimental effect on the structure of macromolecules such as proteins, lipids, and nucleic acids [2].

Cell cultures are particularly useful for studying the effects of oxidative stress on cells. Adding hydrogen peroxide is a method commonly used to induce oxidative stress in cells [3]. Cells incubated with pro-oxidant compounds constitute great models of inflammation. Such models help explain the role and pathogenesis of oxidative stress in many diseases, e.g. in neurodegenerative disorders, and rheumatoid arthritis. In recent years, naturally occurring antioxidants like vitamin E, flavonoids, and polyphenols have been used in oxidative stress research due to their beneficial effects on reducing ROS in cells. However, researchers are still looking for other compounds that have antioxidant properties. [4].

This study aimed to determine the impact of different concentrations of the pro-oxidant H_2O_2 on the occurrence of oxidative stress and the resulting changes in the cell metabolome. Additionally, the study examined the antioxidant potential of curcumin and metformin on cell lines. Using cellular activity assays: MTT and DCF-DA, along with metabolomic analysis (1H NMR), studies examined how different concentrations of hydrogen peroxide affect cell viability and the number of free radicals in the HEK-293 cell line. These studies also looked at changes in the cells' metabolome. Metabolomics studies (LC-MS and NMR) confirmed that hydrogen peroxide significantly affects the cellular metabolome, causing oxidative damage in mitochondria as part of the Krebs cycle. In subsequent studies, appropriate H_2O_2 concentrations initiating the appearance of oxidative stress on cell line and

appropriate concentrations of therapeutic agents were selected. These studies confirmed the antioxidant properties of curcumin and metformin.

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FP32

Exploring Triazine-Based Dual Ligands Targeting 5-HT$_6$ Receptor and FAAH Enzyme: A Promising Approach for Alzheimer's DiseaseTherapy

Kinga Czarnota-Łydka[a,b*], Grzegorz Satała[c], Leonardo Brunetti[d], Luca Piemontese[d], Antonio Laghezza[d], Katarzyna Kucwaj-Brysz[a], Antonio Carrieri[d], Jadwiga Handzlik[a]

[a] Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University, Medical College, Medyczna 9, PL 30-688 Kraków, Poland

[b] Doctoral School of Medical and Health Sciences, Jagiellonian University Medical College, św. Łazarza 15, 31-530 Krakow, Poland

[c] Department of Medicinal Chemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

[d] Department of Pharmacy-Drug Sciences, University of Bari Aldo Moro, Via Orabona 4, 70125, Bari, Italy

*kinga.czarnota@doctoral.uj.edu.pl

Alzheimer's disease (AD) is a devastating neurological condition without a cure, affecting about 50 million people worldwide. It primarily involves the breakdown and harm to synapses, crucially contributing to the emergence and progression of both its behavioral and physiological symptoms. Alongside the well-known cholinesterases (AChE and BChE), the serotonin 5-HT$_6$ receptor (5-HT$_6$R) within the central nervous system (CNS) and fatty acid amide hydrolase (FAAH), a significant enzyme in the endocannabinoid system, are being explored as potential therapeutic targets for AD. To combat this neurodegenerative disease, there's a growing focus on multitarget compounds. Hence, this study introduces a range of dual ligands that simultaneously target the serotonin 5-HT$_6$ receptor and FAAH enzyme, offering promise in the quest for new therapeutic solutions.

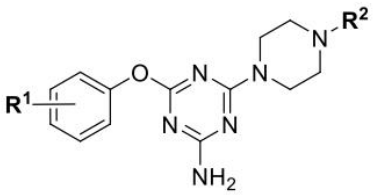

Fig. 1. General structure of the investigated compounds.

Therefore, with the support of molecular modeling, a series of 1,3,5-triazine-based derivatives (Fig. 1.) were designed and synthesized, as a potential dual 5-HT$_6$R/FAAH ligands. New compounds were obtained in multi-step synthesis involving N-alkylation, nucleophilic substitution and reductive amination. Then, biological studies to determine the affinity for the 5-HT$_6$R in radioligand binding assays and an inhibition potency for FAAH in enzymatic assays were carried out.

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FP33

Targeting Na+/Li+/Ca2+ exchanger with benzodiazepine analogues to restore apoptosis in different cancer cells

Daniela Secci,[a] Ivan Džajić,[a] Marzia Fois,[a] Maxime Guéguinou,[b] Luis A. Pardo,[c] Andrej Emanuel Cotman,[a] Tihomir Tomašič,[a] Lucija Peterlin Masic[a]

[a]Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
[b]UMR INSERM 1069 Nutrition Croissance et Cancer (N2C), Université de Tours, Faculté de Médecine, 10 Boulevard Tonnellé 37032 Tours, France
[c]Max-Planck Institute for Experimental Medicine, AG Oncophysiology, Hermann-Rein-Str. 3, Göttingen, 37075, Germany

daniela.secci@ffa.uni-lj.si

Mitochondria are membrane-bound organelles that produce energy in eukaryotic cells. They are crucial in a variety of physiological functions, including calcium signaling, cell growth and differentiation, cell cycle regulation, and cell death [1]. Alterations in mitochondrial function are frequently associated with disease, including cancer. Notably, variations in mitochondrial Ca^{2+} ($mtCa^{2+}$) have recently emerged as a distinctive characteristic of cancer cells [2]. Calcium homeostasis is primarily controlled by the balance of Ca^{2+} uptake by MCU (calcium uniporter complex) and Ca^{2+} extrusion modulated by NCLX ($Na^{+}/Li^{+}/Ca^{2+}$ exchanger). Considering their critical importance, MCU and NCLX channels are druggable targets in cancer treatments. However, the contribution of NCLX in cancer biology has yet to be extensively studied. Despite this, the mitochondrial Na^{+}/Ca^{2+} exchanger was targeted utilizing its inhibitor CGP37157 (CGP), a benzodiazepine derivative, and it was found to promote mitochondrial damage and apoptosis induction in various cancer cell lines [3][4]. Considering this information, we proceeded with the design and synthesis of different CGP analogues with the benzodiazepine scaffold, a mitochondria-targeting moiety (MTM), and a linker. Preliminary testing of the recently synthesized compounds showed notable IC_{50} values in the micromolar range and the ability to induce apoptosis of Colo-357 cells. Additionally, positive results from activity studies on HCT116 KO WT and HCT116 KO NCLX suggested that the compounds might preferentially inhibit the mitochondrial NCLX channels.

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FP34

Impact of D2AAK1 and its derivatives on HT-22 neuronal cell line

Piotr Wójcik[a], Michał Jastrzębski[a], Agnieszka Kaczor[a]

[a] Department of Synthesis and Chemical Technology of Pharmaceutical Substances,

Faculty of Pharmacy with Division of Medical Analytics, Medical University of Lublin

4A Chodzki St., PL-20093 Lublin, Poland

piotr.wojcik2@umlub.pl

In recent years, neurodegenerative diseases have become an increasingly serious problem for the healthcare system, and counteracting them has become a target for many scientists. For this purpose, our team synthesized a new compound, D2AAK1, and its derivatives.

Our previous research suggests that D2AAK1 has cytoprotective as well as pro-proliferative properties . To obtain greater effectiveness, we prepared a number of derivatives and compared their effects with D2AAK1.

To assess the effectiveness of the compounds, we used the resazurin assay. This method is based on the reduction of oxidized, non-fluorescent blue resazurin to a red fluorescent dye (resorufin) by the mitochondrial respiratory chain in live cells. The amount of resorufin produced is directly proportional to the number of living cells. To investigate the mechanism of action of D2AAK1, the expression of certain proteins involved in the regulation of apoptosis/cytoprotection was also measured using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). In this method, the amplification of a targeted cDNA molecule (which corresponds to the expression of the targeted protein RNA) is measured in real time during PCR.

Our results show that D2AAK1 may act through the activation of cytoprotective pathways involving the anti-apoptotic Bcl2 and antioxidant HO-1 proteins, as it increases their expression. Moreover, high activity of both D2AAK1 and its derivatives was observed using the resazurin method at concentrations of 1-10 µM. After treatment with D2AAK1, cell viability increased by 40% compared to the control, and some derivatives were even more potent, leading to a 60% higher viability. Therefore, D2AAK1 is a promising compound for the treatment of diseases involving a decrease in the number of neurons, such as neurodegenerative diseases.

The research was funded by the National Science Centre, Poland (2021/43/B/NZ7/01732).

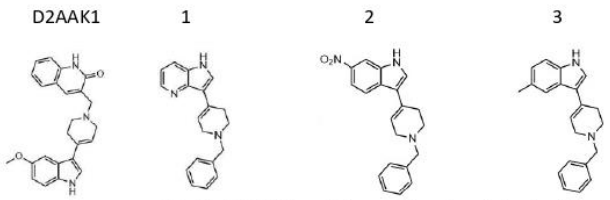

Fig. 1. D2AAK and its most potent derivatives.

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Poster communication

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PC1

NEW QUINOLINONYL DERIVATIVES AS SARS-CoV-2 NSP13 INHIBITORS

Albano, A.;[a] Madia, V. N.;[a] Ruggieri, G.;[a] Ialongo, D.;[a] Patacchini, E.;[a] Arpacioglu, M.;[a] Messore, A.;[a] Scipione, L.;[a] Corona, A.;[b] Esposito, F.;[b] Amatore, D.;[c] Faggioni, G.;[c] De Santis, R.;[c] Lista, F.;[c] Tramontano, E.;[b] Di Santo, R.;[a] and Costi, R.[a]

[a] *Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, "Sapien-za" Università di Roma, p.le Aldo Moro 5, I-00185 Rome, Italy;* [b] *Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, SS554 -09042 Monserrato (CA) Italy;* [c] *Army Medical Center, Scientific Department, p.za Celimontana 50, Rome 00184, Italy.*
aurora.albano@uniroma1.it

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent behind the 2019 global coronavirus pandemic (COVID-19). Even though successful vaccination programs to counteract COVID-19 are available worldwide, little has been accomplished in the development of antivirals to treat the disease. The disparity in COVID-19 vaccination coverage, vaccine resistance, the emergence of SARS-CoV-2 variants of concern, the increased transmission ability and the potential to evade both vaccination and acquired immunity emphasize the relevance of developing antiviral drugs to treat SARS-CoV-2 infections.[1] The development of new antiviral agents is, therefore, of utmost importance.

The SARS-CoV-2 non-structural protein 13 (nsp13) has been identified as a promising drug target for the development of antivirals due to its pivotal role in viral replication. The CoVs nsp13 is a multidomain enzyme of 601 amino acids that targets the natural nucleotides and deoxynucleotides as substrates when performing its adenosine triphosphatase (ATPase) activity, utilizing the energy of nucleotide triphosphate hydrolysis to unwind DNA or RNA with a 5'-3' polarity.[2] Moreover, nsp13 is the most conserved non-structural protein within the coronavirus family, with a 100% of sequence similarity between SARS-CoV-1 and SARS-CoV-2 nsp13 enzymes.[3] Several compounds have been reported to inhibit SARS-CoV-1 nsp13 and, very recently, also SARS-CoV-2 nsp13 has been actively explored as drug target, with some reports describing small molecules as inhibitors of SARS-CoV-2 nsp13. Among them, aryl diketo acids (DKAs), previously described as inhibitors of SARS-CoV-1 nsp13, have been reported as inhibitors also of SARS-CoV-2 nsp13.[4] On the other hand, the DKA chain suffers from several limits related to the pharmacokinetic and pharmacodynamic profiles. Therefore, to overcome the limits of the DKA moiety, a variety of compounds were developed by transferring the DKA chain to scaffolds characterized by improved druglike qualities. Therefore, we carried out a semi-random screening on our in-house library of non-DKA derivatives, previously developed to deal out the undesirable DKA properties, identifying a promising hit compound as micromolar nsp13 inhibitor.

We synthesized a set of derivatives structurally correlated with the hit, obtaining a new series of dual inhibitors of both the SARS-CoV-2 nsp13-associated activities. The data coming from the biological assays will be shown and discussed.

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PC2

Design and Synthesis of AKT1 Allosteric Inhibitors for Acute Myeloid Leukemia.

M. Casciari,[a] M. Pacetti,[a] E. Primavera,[a] F. Milano,[b] A. Astolfi,[a] V. Cecchetti,[a] M.P. Martelli,[b] M.L. Barreca,[a] and S. Massari[a]

[a] Department of Pharmaceutical Sciences, University of Perugia

Via del Liceo 1, 06123 Perugia, Italy

[b] Hematology and Clinical Immunology, Department of Medicine and Surgery,

University of Perugia

Piazzale Gambuli 1, 06129 Perugia, Italy;

marta.casciari@gmail.com

The serine/threonine kinase AKT is a critical component of the PI3K/AKT/mTOR signaling pathway, whose hyperactivation is implicated in the development of many human cancers and resistance to chemotherapy.[1] Several studies have highlighted a correlation between the constitutive activation of PI3K/AKT/mTOR and acute myeloid leukemia (AML). AML is a heterogeneous hematopoietic malignancy characterized by an abnormal proliferation of myeloid progenitor cells, and, while rare, its prognosis is globally poor. Very recently, our research group has focused on developing AKT1 inhibitors for AML treatment. We identified a compound, 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**T126**), which exhibits an IC_{50} of 1.99 ± 0.11 µM and a ligand efficiency (LE) of 0.35. Further analysis of **T126** revealed its capacity to bind the AKT1 ATP-binding pocket, inhibiting cell growth and inducing apoptosis in a panel of AML cells at low micromolar concentrations.[2]

In this work, focusing on the identification of targetable AKT1 allosteric inhibitors, we generated a pharmacophore model by exploiting potent AKT1 allosteric inhibitors reported in the literature. Then, by combining the pharmacophore features with the core of **T126**, we synthesized a set of small molecules as AKT1 allosteric inhibitors. Among these, one exhibited a particularly promising profile, showing an IC_{50} in the low micromolar range and displaying strong inhibitory activity against two mutated AML cell lines compared to the wild type.

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PC3

Insights into the Development of Isoform-Selective, Non-Interfering NOX Inhibitors

Mariana Castelôa[a], Joana Reis[b,c], Carla Lima[a], Marta Massari[b], Sara Marchese[b], Lorenzo Basile[b], Fernanda Borges[a], Sofia Benfeito[a], Andrea Mattevi[b], Daniel Chavarria[a]

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

[b] Department of Biology and Biotechnology Lazzaro Spallanzani, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

[c] Department of Cancer Biology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA, USA

up201606405@edu.fc.up.pt

Oxidative stress has been implicated in several pathologies and emerges when the balance between the generation of reactive oxygen species (ROS) and the cells' ability to clear them is lost. One of the main mechanisms contributing to excessive ROS production is the increased activity of NADPH oxidases (NOXs), a family of 7 multi-subunit enzymes. As a result, NOXs are considered potential therapeutic targets to treat oxidative stress-related disorders. However, the available NOX inhibitors usually exhibit several drawbacks, including significant assay-interfering effects, low inhibition potency, high cytotoxicity, and lack of specificity and/or isoform selectivity [1]. VAS2870 is a non-interfering NOX inhibitor that binds covalently to a conserved cysteine residue present in the NOX dehydrogenase domain. However, like other NOX inhibitors, it exhibits low inhibition potency and selectivity, poor aqueous solubility, and cytotoxicity [2].

In this work, we designed and synthesized a small library of VAS2870-based compounds to potentially obtain a NOX inhibitor with improved potency, selectivity, and safety, while maintaining the mechanism of action and non-interfering properties of VAS2870. Therefore, a small library of compounds was successfully synthesized and tested on full-length NOX1, NOX2, NOX4, and NOX5. The assay-interfering properties and the stability of the inhibited protein were also evaluated. Overall, an isoform-selective, non-interfering inhibitor was successfully obtained. The results obtained will be presented in this communication.

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PC4

Chemometric tools for the photostability study of tamoxifen in the oral formulation and stabilization strategies

M. Chieffallo, G. Ioele, F. Grande, G. Cirillo, M.A. Occhiuzzi, A. Garofalo, M. De Luca

[a] Department of Pharmacy Health and Nutritional Science, University of Calabria

Via P. Bucci 87036 Rende, Italy

martina.chieffallo@unical.it

One of the major challenges in pharmaceutical and technological research is ensuring the stability of drugs under various conditions, including exposure to light. Protecting the molecular integrity and therapeutic function of active molecules from light-induced degradation is critical during all stages of drug development. The study of the photostability properties of drugs is regulated by the International Conference of Harmonisation (ICH), which specifies the experimental procedures for testing the stability of new drugs.

This study proposes a chemometric methodology coupled with spectroscopy for the analysis of the degradation kinetics of the photolabile antiestrogen tamoxifen (TAM).[1] The degradation experiments were performed on the ethanolic solution of the pure compound and on an oral formulation of the TAM. The Multivariate Curve Resolution - Alternating Least Squares (MCR-ALS) algorithm was applied to elaborate the experimental data obtained by UV/Vis analysis (Figure 1). The resolution of the multiset data from photodegradation experiments under different conditions allowed the study of the behaviour of TAM when photoprotection strategies were tested. The photodegradation of TAM in the oral solution occurred at twice the rate of the ethanolic solution ($k=4.1x10^{-3}$ s^{-1} vs $k=2.2x10^{-3}$ s^{-1}, respectively). Different concentrations of quercetin (QUE) and ascorbic acid (ASC) were mixed in solution and in an oral formulation, so binary mixtures (TAM-QUE and TAM-ASC) and ternary mixtures (TAM-ASC-QUE) were prepared in different proportions with the aim of testing their radical scavenging properties. Experimental data were resolved by augmented analysis which allows to put together all the data matrices. Both quercetin and ascorbic acid in ethanol stabilize tamoxifen. The use of ascorbic acid in a ratio 1:1 (TAM:ASC) reduced the effect of light irradiation by lowering the k value to $9.0x10^{-4}$ s^{-1}.

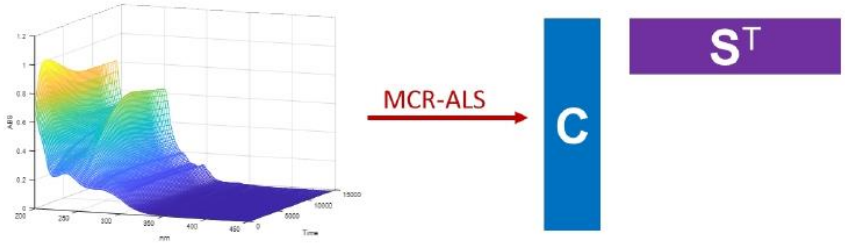

Figure 1. Schematic representation of the chemometric methodology developed for the analysis of TAM degradation.

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PC5

HUMAN NADPH OXIDASES: DISCOVERY AND OPTIMIZATION OF NOVEL INHIBITORS

Beatrice Noce[a], Davide Ciampoli[a], Joana Reis[c], Christoph Gorgulla[c,d], Marta Massari[b], Sara Marchese[b], Sergio Valente[a], Chiara Lambona[a], Lorenzo Basile[b], Ricarda Törner[c], Huel Cox[c], Thibault Viennet[c], Moon Hee Yang[c], Melissa Ronan[e], Matthew Rees[e], Jennifer Roth[e], Lucia Capasso[f], Angela Nebbioso[f], Lucia Altucci[f], Haribabu Arthanari[c], Antonello Mai[a] and Andrea Mattevi[b]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, Rome, Italy

[b] Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy

[c] Department of Biological Chemistry and Molecular Pharmacology, Blavatnik Institute, Harvard Medical School (HMS), Boston, MA, USA; Department of Cancer Biology, Dana-Farber Cancer Institute (DFCI), Boston, MA, USA

[d] Department of Physics, Faculty of Arts and Sciences, Harvard University, Cambridge, MA, USA

[e] Broad Institute, Cambridge, MA, USA

[f] Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

(P.le A.Moro 5, 00185 Rome, Italy)

davide.ciampoli@uniroma1.it

NADPH oxidases (NOXs) generate reactive oxygen species (ROS) and have seven mammalian isoforms linked to diseases like fibrosis and cancer[1,2]. We aimed to identify both covalent and non-covalent NOX inhibitors. We enhanced **VAS2870**, a pan-NOX inhibitor, and explored chemical groups of lead compound **M41** to create more druggable molecules. Using advanced synthesis techniques, we developed triazolopyrimidine and quinoline-based inhibitors and evaluated their NOX activities through various assays. We have designed **VAS2870** analogues, a NOXs pan-inhibitor that forms an adduct with the enzyme, through the covalent alkylation of Cys668, found to reside in the dehydrogenase domain of NOXs[3]. Firstly, we have introduced on the C7 of the triazolopyrimidine different functional groups to explore the possibility to obtain non-covalent inhibitors. Then, we modified the C7 position and replaced the benzoxazoyl group with acrylamide moieties (established as pharmacologically powerful cysteine modifiers)[4]. Secondly, based on the NOX5-DH crystal structure showing FAD near Cys668, we designed a new class of covalent ligands. We used pharmacophore moieties that react with isoalloxazine, inserting propylamide, propargylamide, and propargylamine into the para position of **VAS2870**. **MC4768**, **MC4767**, **MC4762** have given the most promising results in terms of enzymatic inhibition. In addition, observing the presence of the pargyline in the structure of **MC4762**, we tested this compound against MAO-B to find a dual inhibitor (NOX2/MAO-B). The second project originated from **M41**, a compound obtained through virtual screenings, which exhibited an increase in the inflection temperature of 3.6±0.1°C on NOX5-DH. We produced **MC4876**, replacing the 1,4-dioxaspiro with a piperidine group, enhancing its selectivity for NOX2. CETSA confirmed the NOX2 engagement by **MC4876** results. Further, we selected **M41** and **MC4876** to study NOX inhibition in cancer cell models, focusing on the NOX2 role in myeloid cells using the U937 cell line, confirming their sensitivity to **M41** and **MC4876**. Our work advances NOX inhibitor development, offering valuable tools for studying ROS-producing enzymes' chemical biology and pharmacology.

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PC6

Novel indole derivatives as potent CYP17A1 inhibitors

Angelika Grudzińska[a] Amit V. Pandey[b], Jibira Yakubu[b], Flemming Steen Jørgensen[c], Fredrik Björkling[c], Tomasz M. Wróbel[a]

[a]Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University of Lublin, 20-093 Lublin, Poland

[b]Department of Biomedical Research, University of Bern, 3010 Bern, Switzerland.

[c]Department of Drug Design and Pharmacology, University of Copenhagen, DK-2100 Copenhagen, Denmark

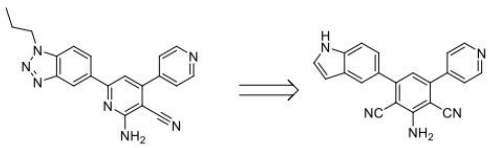
angelika.grudzinska@umlub.pl

Introduction: Prostate cancer is one of the most common cancers among men worldwide. Treatment of hormone-sensitive prostate cancer has limitations and numerous sides effects. Over time, disease progresses to castration-resistant prostate cancer (CRPC). One of the strategies for treating CRPC is to block the CYP17 enzyme. So far, a steroidal drug compound, abiraterone, is the only clinically used CYP17 inhibitor. One of the current perspectives is focused on the discovery and the evaluation of non-steroidal CYP17A1 inhibitors.[1]

Aim of the study: Synthesis and evaluation of novel derivatives based on previously identified virtual hit **1**.[2]

Materials and methods: In chemical synthesis commercially available reagents were used. In biological assays, prostate cancer cell lines LNCaP and human cell lines H295R were used. Antiproliferative activity, % inhibition and IC_{50} of the compounds were determined.

Results: Several analogs based on virtual hit **1** were envisioned to explore structure-activity relationship (SAR). Throughout the synthesis, an unexpected outcome occurred leading to unplanned compounds with markedly increased activity compared to those of the initial design. Consequently, ten novel compounds were synthesized and JCMH-0044 has found out to be the most potent in this series. Subsequently, next series of simplified analogues were synthesized to determine the most preferable geometry of structures and substituent effects. SAR showed that a non-planar geometry as preferred for the activity of the compound. The lack of substituents in the benzene ring is associated with reduced activity.



1 Hit compound
IC_{50} = 130 nM

JCMH0044
IC_{50} = 660 nM

Conclusion: Our studies resulted in a potent CYP17A1 inhibitor. The synthesis and evaluation of analogues of **1** yielded valuable insights for optimizing this class of compounds, potentially advancing the development of potent CYP17 inhibitors as promising candidates for prostate cancer treatment.

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PC7

From magic bullets to nanomedicine: where do we stand?

Eleonora D'Intino[a], Jacopo Forte[a], Maria Gioia Fabiano[a], Federica Rinaldi[a], Maria Carafa[a], Carlotta Marianecci[a]

[a] Department of Chemistry and Technologies of Drugs

P. le Aldo Moro 5, 00185 Rome, Italy

eleonora.dintino@uniroma1.it

Targeted drug delivery (TDD) is an advanced version of Paul Ehrlich's "magic bullet" concept (1907). In fact, it allows the direct delivery of the drug into its targeted body area to overcome off-target accumulation in the body related to the non-specificity of conventional drug delivery, increasing the bioavailability of the delivered drug, consequently reducing the amount of drug required for therapeutic efficacy [1].

Niosomes (NIOs) are promising nanoscale drug carriers composed mainly of surfactants. Like liposomes, they are stable and able to encapsulate both hydrophilic and lipophilic drugs, minimizing their degradation or inactivation after administration. The advantages in designing NIOs are less costs and more stability of surfactants instead of phospholipids composing liposomes. For this reason, NIOs may represent efficient and versatile therapeutic platform treatments. Due to the long production times and the high consumption of reagents, there was the need to develop a new production technique, such as microfluidics, that allows to process small amount of fluids but with the need to evaporate the organic solvent used, introducing purification methods that could reduce the amount of drug entrapped. Thanks to the fine control of the operating parameters, the reproducibility of the processes and low production times, it could be used to scale up from lab to full production. Additionally, the improved repeatability and reproducibility of the results could be used to obtain standardized information about nanocarriers characterization in the perspective of a marketing authorization [2]. The aim of this study was to design, prepare and characterize NIOs composed by Tween 21 and cholesterol by using two different preparation techniques: "Thin Layer Evaporation" (TLE) and "Microfluidic Technique" (MT), to investigate their influence on NIOs hydrodynamic diameter, polydispersity index and ζ-potential. Additionally, NIOs stability and entrapment efficiency (E.E.) of two different probes were also investigated. In conclusion, the obtained results showed that the two preparation methods do not cause any changes in size and bilayer features of the obtained NIOs. NIOs obtained by MT or TLE are similar and able to load both hydrophilic and hydrophobic probes. Even if there is not a clearly better technique because each method has intrinsic advantages and disadvantages, the MT produces much more yield in shorter periods of time so it could represent a new approach to produce nanomedicines in the context of industrial scale-up.

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PC8

Investigating the role of some antitubercular drugs in modulating MmpL3-TMM binding

Michela Guida[a,b], Abraham O. Oluwole[b,c], Jani Bolla[b,d], Carol V. Robinson[b,c], Giovanna Poce[a]

[a] Department of Chemistry and Technologies of Drug, Sapienza University of Rome, 00185 Rome, Italy.

[b] The Kavli Institute for Nanoscience Discovery, Oxford, South Parks Road, Oxford, OX1 3QU, UK.

[c] Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK.

[d] Department of Biology, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK.

michela.guida@uniroma1.it

Antibiotic resistance is widely recognised as one of the main global health problems that we must face: there is an urge for new antibiotics to combat the growing threat of resistant bacteria. Here, we employ native Mass Spectrometry (MS) to gain further understanding on the interaction of some antitubercular drugs with their target protein.

Tuberculosis (TB) is a highly infectious disease caused by Mycobacterium tuberculosis (Mtb). It is the 13th leading cause of death worldwide, with an estimated 10 million people falling ill and 1.3 million deaths in 2022 [1]. The insurgence of antibiotic resistance strains of Mtb makes it a priority to look for new drugs and understand how they work. Trehalose Monomycolate (TMM) is an important component of the mycobacterial cell wall, which plays an important role in the structure and survival of bacteria. It is a complex molecule composed of trehalose, a sugar, and a mycolic acid, a long-chain fatty acid. Mmpl3 (Mycobacterial membrane protein Large 3) is a transmembrane protein that transports TMM from the cytoplasm to the periplasm, where it is further processed and incorporated into the cell wall.

Using native MS, I investigated the interaction between Mtb MmpL3 and TMM, and studied how a series of antitubercular drugs, BM212 [2], BM635, BM859 and BM850 [3], known to target MmpL3, affect it. It was possible to evaluate how the protein binds to the substrate and how the drugs negatively affect the binding. This work confirms that the drugs interact with MmpL3 and suggests that their antitubercular effect is due to their impact on the binding to TMM, essential for the construction of bacterial wall cells.

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PC9

Advancements in Synthesis and Property Evaluation of D2AAK1 Derivatives: A Step Forward in Neurodegenerative Disease Treatment

Michał K. Jastrzębski,[a] Piotr Wójcik,[a] Tomasz Wróbel,[a,] Tadeusz Karcz,[b] Agnieszka Olejarz-Maciej,[b] Piotr Drączkowski,[a] Agnieszka A. Kaczor[a,c]

[a]Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Laboratory, Faculty of Pharmacy, Medical University of Lublin, 4A Chodzki St., PL-20093 Lublin, Poland; [b]Department Of Medicinal Technology and Biotechnology, Faculty of Pharmacy, Jagiellonian University, 9 Medyczna St., PL-30688 Cracow, Poland; [c]School of Pharmacy, University of Eastern Finland, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland

michal.jastrz1998@gmail.com

Neurodegenerative diseases like Alzheimer's present major global healthcare challenges. Searching for new active compounds plays a crucial role in developing effective therapies for these disorders. These efforts seek compounds that can alter disease progression, relieve symptoms, and potentially halt or reverse neurodegeneration. Thus, the pursuit of new compounds through research and synthesis is essential for addressing neurodegenerative diseases and enhancing the quality of life for those affected.

The aim of the research was to synthesize derivatives of the virtual hit D2AAK1. These derivatives - according to preliminary *in silico* studies - possess properties influencing processes related to the pathomechanisms of neurodegenerative diseases. Additionally, the research aimed to investigate the obtained derivatives in vitro.

A series of derivatives of the D2AAK1 compound were obtained. In vitro studies revealed that the obtained compounds are potent MAO-B and AChE inhibitors. Additionally, the effect of D2AAK1 derivatives on the viability of neuronal cells was investigated, revealing significant pro-proliferative activity of the obtained compounds.

Obtaining and studying new compounds that may aid in the treatment of neurodegenerative diseases represents a significant challenge in contemporary medicinal chemistry. In this study, we present the results of the synthesis and investigation of a series of derivatives of the D2AAK1 compound, which possess pro-proliferative properties and affect the enzymatic activity of AChE and MAO-B. A further step forward was taken towards optimizing the structure, and it was demonstrated that the postulated *in silico* properties are confirmed in *in vitro* studies. Further research is necessary to obtain derivatives with the optimized multi-target activity profile.

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PC10

Development of *in vivo* efficacious AURKA-degraders for the treatment of neuroblastoma

Simon Krols[1,3], Muhammad Rishfi[2,3], Pieter-Jan De Sutter[4], Fien Martens[2,3], Martijn Risseeuw[1,3], Ellen Sanders[2], Aline Eggermont[2], Fanny De Vloed[2], Joshua Robert Goulding[2], Jan Molenaar[5], Frank Speleman[2,3], Bram De Wilde[2,3], An Vermeulen[4], Kaat Durinck[2,3] and Serge Van Calenbergh[1,3]

[1]Laboratory of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ottergemsesteenweg 460, Ghent University, Belgium
[2]Department of Biomolecular Medicine, Ghent University, Belgium
[3]Cancer Research Institute Ghent (CRIG), Ghent, Belgium
[4] Laboratory of Medical Biochemistry and Clinical Analysis, Department of Bioanalysis, Ghent University, Belgium
[5] Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

Simon.Krols@UGent.be

Aurora kinase A (AURKA) inhibition is actively being pursued as a treatment for the paediatric cancer neuroblastoma (NB). However, current inhibitors trigger protein upregulation, which may drive the emergence and survival of drug-resistant cells. In contrast to inhibition, AURKA degradation may overcome upregulation and additionally target AURKA's scaffolding functions, potentially resulting in a more pronounced growth inhibitory effect. Our efforts in synthesizing AURKA-targeting degraders have led to the discovery of a state-of-the-art PROTAC SK2188. SK2188 induces potent ($DC_{50,24h}$ = 4 nM), fast, profound ($D_{max,24h}$ 89%) and selective AURKA degradation. Furthermore, compared to inhibition, AURKA degradation resulted in up to 10 fold stronger cell growth inhibition in neuroblastoma cell lines and patient-derived organoids. Despite these promising results, high *in vivo* clearance for SK2188 hampered its *in vivo* evaluation. Further structural optimization was carried out and resulted in potent degrader SK4454 (DC_{50}: 4 nM, D_{max}: 94%). SK4454 showed a favorable PK profile with free drug concentrations sufficiently covering the DC_{50} value. Finally, SK4454 strongly reduced tumor AURKA levels (80% and 73% reduction after 4 and 8h respectively) in mice with a neuroblastoma IMR-32 xenograft, thereby providing proof-of-concept of *in vivo* AURKA degradation.

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PC11

Metabolic Consequences of ESCRT-I Deficiency: A NMR Spectroscopy Study

Patryk Kuropka[a], Jarosław Cendrowski[b], Natalia Pudełko-Malik[a], Piotr Młynarz[a]

[a] Department of Biochemistry, Molecular Biology and Biotechnology, Faculty of Chemistry, Wroclaw University of Science and Technology
Norwida 4/6, 50-373 Wrocław, Poland

[b] Laboratory of Cell Biology, International Institute of Molecular and Cell Biology
Ks. Trojdena 4, 02-109 Warsaw, Poland

patryk.kuropka@pwr.edu.pl

Metabolomics, an unbiased approach for examining small molecules within biological matrices, is pivotal for understanding metabolic networks. NMR spectroscopy enables robust metabolite profiling, offering insights into biochemical pathways and potential therapeutic targets, therefore aiding in the development of novel therapeutics.

The Endosomal Sorting Complex Required for Transport (ESCRT) plays an important role in cellular membrane dynamics, protein sorting, and degradation, vital for numerous physiological processes such as cell division and receptor signalling. Recent studies have begun to implicate ESCRT proteins, including components of ESCRT-I subcomplex, in the regulation of cellular metabolism, suggesting a potential interplay between membrane dynamics and metabolic pathways.

Using NMR spectroscopy, we analysed extracellular and intracellular metabolic profiles of HEK293 cells with deficiency of ESCRT-I due to removal of its core components TSG101 or VPS28 proteins, as compared to control cells. Methanol-extracted cell and media samples were analysed using 1D NOESY water suppression on a Bruker Avance II spectrometer. Spectral data processing involved phasing, baseline correction, and metabolite identification with Chenomx software, followed by PQN normalization, COW alignment, and univariate statistical analysis in MATLAB.

According to the results of NMR analysis cells lacking ESCRT-I have elevated glucose and glutamine consumption as well as increased lactate release, hallmarks of Warburg effect. Further results obtained using various cellular and molecular assays suggest that the metabolic reprogramming, identified by NMR, is a result of impaired utilization of lysosome-derived nutrients, such as lipids and branched chain amino acids, culminating in a shift towards glycolytic metabolism and metabolic adaptation to ESCRT dysfunction. The analysis results reveal the broader physiological significance of ESCRT beyond membrane trafficking and sheds light on its potential involvement in modulating metabolic pathways and maintaining cellular homeostasis.

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PC12

Innovative Dual Selective HDAC1,2/LSD1 Inhibitors to Fight Cancer

Chiara Lambona [a], Beatrice Noce [a], Elisabetta Di Bello [a], Carola Castiello [a], Andrea Mattevi [b], Angela Nebbioso [c], Lucia Altucci [c], Sergio Valente [a], and Antonello Mai [a]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome
P. le A. Moro 5 00185 Rome (Italy)

[b] Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia
Via A. Ferrata 9 27100 Pavia (Italy)

[c] Department of Medicine of Precision, University of Campania "Luigi Vanvitelli"
Vico L. De Crecchio 7 80138 Naples (Italy)

chiara.lambona@uniroma1.it

Nowadays a simultaneous inhibition of two or more targets involved in cancer can improve therapeutic efficacy over a single-target inhibitor [1]. In collaboration with the Cole's and Mattevi's team, we previously developed Corin, an hybrid molecule that targets the CoREST complex resulting in the inhibition of LSD1 and HDAC1 at sub-micromolar doses [2]. The aim of our present research is to develop, synthesize and validate anticancer activity of new GSK2879552-based hybrids, which could specifically inhibit the specific isoforms HDAC1 and/or HDAC2, in addition to LSD1. Particularly, the carboxylic acid of GSK-2879552, an LSD1 inhibitor undergoing clinical trials (NCT02929498, NCT02034123, NCT02177812), was modified by incorporating the *ortho*-aminoanilide portion of Entinostat, an inhibitor of HDAC 1-3. The resulting compound **1a** was primarily obtained. Later, the amino group in the *para* position of the *o*-aminoanilide portion was replaced with different substituents including a fluorine atom (**1b**) to study hydrogen bonding effects, a thiophene (**1c**) known to confer selectivity to HDAC1/2 [3], and various aromatic monocyclic groups (**1d-m**) isomers/bioisosters of the 2-thienyl group. Our preliminary results proved that the novel hybrid compounds **1c**, **1h** and **1m** displayed nanomolar inhibition against HDAC1 and HDAC2 and sub-micromolar inhibition against LSD1. These three most promising hybrid compounds were tested in typologically different cancer cell lines (SKMEL28, U937, HL60, A549, HCT-116 and MCF7) and the dual inhibitor **1c** displayed a higher cell vitality reduction than the reference inhibitor Corin, with the A549, HCT-116 and MCF7 cell lines being the most responsive ones. During the next months we will perform further in-depth biochemical and biological assays in one of the most sensitive cell lines aforementioned.

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PC13

Design, synthesis and biological evaluation of multitarget hybrid molecules containing NHC-Au(I) complexes and carbazole moieties

Maria Marra[a], Domenico Iacopetta[a], Jessica Ceramella[a], Annaluisa Mariconda[b], Assunta D'Amato[c], Rubina Troiano[c], Alessia Catalano[d], Carmela Saturnino[b], Camillo Rosano[e], Stefano Aquaro[a], Pasquale Longo [c], Maria Stefania Sinicropi[a]

[a] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, via Pietro Bucci, 87036 Arcavacata di Rende, Italy;
[b] Department of Science, University of Basilicata, 85100 Potenza, Italy;
[c] Department of Chemistry and Biology "A. Zambelli", University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy;
[d] Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy;
[e] Proteomics and Mass Spectrometry Unit, IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, 16132 Genova, Italy;
mariamarra1997@gmail.com

N-heterocyclic carbenes (NHCs) represent suitable ligands for rapid and efficient drug design because they offer the advantage of being easily chemically modifiable and are able to bind various substituents, including transition metals such as gold [1]. NHC-gold complexes were proven to possess several biological properties, amongst them anticancer activity [2]. Besides, carbazole derivatives demonstrated anticancer, antibacterial, anti-inflammatory, anti-psychotropic and other activities. Herein, we report the design, synthesis and biological evaluation of a series of new hybrid molecules in which NHC-Au(I) complexes and *N*-alkylthiolated carbazoles [3] are linked together. The identified leads were found to possess anticancer, anti-inflammatory and antioxidant properties, with a high potential as multitarget agents.

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PC14

From Iloperidone to new Sigma1 agonists: a structre-based approach for Huntington's disease treatment

Elisa Patacchini[a], Davide Ialongo[a], Valentina Noemi Madia[a], Antonella Messore[a], Andrea Ilari[b], Sandro Cosconati[c], Roberto Di Santo[a], Roberta Costi[a].

[a]Institute Pasteur - Cenci Bolognetti Foundation, Department of Medicinal Chemistry and Technology, "Sapienza" University of Rome, p.le Aldo Moro 5, 00185 Rome, Italy;
[b]Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania "Luigi Vanvitelli", 81100 Caserta, Italy;
[c]Institute of Molecular Biology and Pathology (IBPM), National Research Council of Italy (CNR), 00185 Rome, Italy.

elisa.patacchini@uniroma1.it

Huntington's disease (HD) is an autosomal dominant disorder caused by a mutation in the HTT gene, which progressively leads neurons in parts of the brain to break down and die. Unfortunately, to date there are no effective treatments that can stop or prevent the onset of this devastating disease. However, recent and growing numbers of studies are showing how the sigma-1 receptor (σ1R) may be implicated in the control of several neurodegenerative disorders, including HD[1]. The σ1R is a small membrane receptor expressed in the central nervous system, whose 3D structure has been recently determined by X-ray crystallography. Substantial evidence has shown that agonists have neuroprotective activity in neurodegenerative diseases. Nevertheless, the structural basis for agonism or antagonism on σ1R is largely unknown. In general, the overall conformation of the receptor bound to the agonist crystallizes similarly to that bound to the antagonist, except for a shift of about 1.8Å in the α4 helix[2]. Probably, this shift is responsible for the tendency of agonists to decrease the oligomeric state of the protein and can be used as a discriminator for classification into agonist. Through structure-based computational methods, we designed new Iloperidone analogues as potential σ1R agonists. Indeed, very recently, a high binding affinity for σ1R of the antipsychotic Iloperidone has been demonstrated[3,4]. From our computational studies, including cross-docking procedures and molecular dynamics simulations[5], the pharmacophoric groups have emerged. In detail, the most stable interactions are established by the nitrogen atom of the piperidine ring of Iloperidone, which is positively charged at physiological pH. This charge allows the molecule to interact with the Phe107 of protein and the negatively charged Glu172 residue. Starting to these data, the chemical structure of this antipsychotic drug has been modified applying a scaffold hopping approach, to obtain a pronounced agonist of the σ1R. We synthesized new small molecules that retained the piperidine core and replacing the benzoisoxazole ring (responsible for a generic π-π interaction) with oximes. Then, we functionalized the oxygen atom of the oxime group, to increase the steric encumbrance between α5 and α4 helixes, with shifts of the latter. Data will be shown and discussed.

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PC15

Structure-based pharmacophore modelling, computational alanine scanning, virtual screening, and simulation studies for the identification of potential elastase inhibitors with anti-aging activity

Giovanna Pitasi[a], Andrea Brancale[b], Laura De Luca[a]

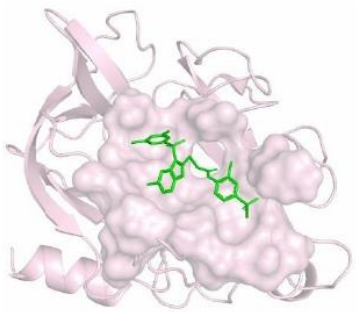
[a] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres 31, I-98125 Messina, Italy

[b] Department of Organic Chemistry, University of Chemistry and Technology, Prague, 16628. Prague, Czech Republic

giovanna.pitasi@studenti.unime.it

Skin aging is characterized by wrinkles, fine lines, loss of elasticity and uneven pigmentation. The main hallmark of skin ageing is the loss of extracellular matrix (ECM) structure,[1] which represents the outermost component of the skin and acts as a barrier to external stimuli. ECM is composed of various components, such as laminin, collagen, elastin, and hyaluronic acid. Elastin gives elasticity to the skin and is broken down by elastase, a protein belonging to the chymotrypsin family. Elastase is involved in the degradation of ECM components such as collagen, fibronectin, and other ECM proteins. Therefore, elastase inhibitors can prevent ECM degradation.[2]

In this regard, we present a computational study to search for potential inhibitors of elastase enzyme. A refined structure-based pharmacophore was created using a combination of pharmacophore modelling, computational alanine scanning and molecular dynamics. Virtual screening of synthetic and natural compounds was carried out, followed by molecular docking studies and calculations of the binding free energy. The result was a selection of the most promising compounds that were subjected to molecular dynamic simulations.



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PC16

Estimation of passive gastrointestinal absorption of a novel thiophene/hydrazones as dual COX-2 and 5-LOX inhibitors using PAMPA test

Vladimir Dobričić[a], Milkica Crevar[a], Jelena Bošković[a], Zafer Sahin[b], Seref Demirayak[c], Goknil Pelin Coskun[d]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy - University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
[b] Department of Chemistry, Emory University, 30322, Atlanta, Georgia. USA
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul Health and Technology University, 34275, Istanbul, Turkiye
[d] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Acibadem Mehmet Ali Aydinlar University, 34752 Istanbul, Turkiye
milkica.crevar@pharmacy.bg.ac.rs

Passive gastrointestinal absorption of three dual COX-2 and 5-LOX inhibitors that belong to thiophene/hydrazones (**GPS-4**, **GPS-9** and **GPS-10**) was estimated by PAMPA test, using a mixture of hexadecane and hexane as an artificial membrane. For comparison, commercially available COX (mefenamic acid, naproxen, indomethacine) and 5-LOX (zileuton) inhibitors were used. The starting solutions were prepared in phosphate buffer pH 5.5, while the acceptor medium consisted of the phosphate buffer pH 7.4. Concentrations of tested compounds in starting solutions, donor and acceptor medium after incubation were measured by a HPLC-DAD method.

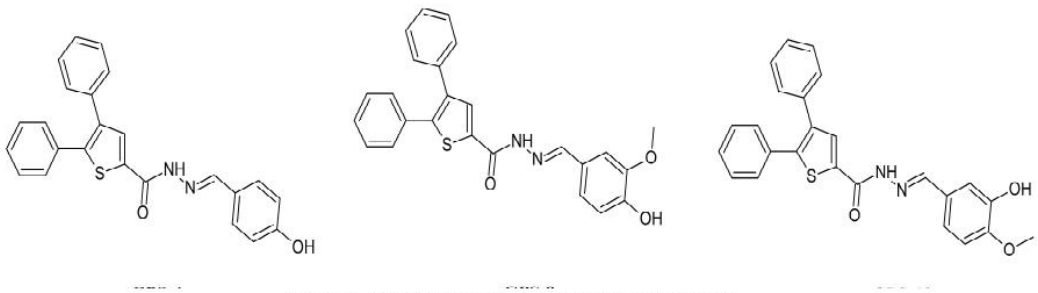

Figure 1. Chemical structures of tested compounds

GPS-9 and **GPS-10** had higher permeability coefficients and higher expected passive gastrointestinal absorption than **GPS-4** (logP values were -4.53, -4.63 and -5.24, respectively). Tested thiophene/hydrazones showed lower permeability coefficients and lower expected passive gastrointestinal absorption than commercially available COX inhibitors (logP values were -3.41 (indomethacine), -3.50 (naproxen), -4.01 (mefenamic acid)), but higher than zileuton (logP was -5.24).

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PC17

Design, synthesis and anticancer activity of novel *first-in-class* dual LSD1/PRMT5 inhibitors in acute myeloid leukemia

Alessia Raucci,[a] Carola Castiello[a], Francesco Fiorentino[a], Luca Pignata[b], Elisa Arceci[c], Elisabetta Di Bello[a], Tiziana Bonaldi[d], Marco Barone[g],Tobias Madl[e], Anand Jeyasekharan[f], Andrea Mattevi[g], Sergio Valente[a], Ernesto Guccione[c], Antonello Mai[a]

[a] Dept. of Drug Chemistry and Technologies, Sapienza University of Rome, P. le A. Moro 5 00185 Rome (Italy)
[b] Dept. of Biochemistry, National University of Singapore, Singapore
[c] Icahn School of Medicine at Mount Sinai, New York, USA
[d] Dept. of Experimental Oncology, European Institute of Oncology (IEO) and Dept. of Oncology and Haematology-Oncology, University of Milan, Milan, Italy
[e] Gottfried Schatz Research Centre for Cell Signalling, Metabolism and Ageing, Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria;
[f] Cancer Science Institute of Singapore, National University of Singapore, Singapore
[g] Dept. of Biology and Biotechnology, University of Pavia, Pavia, Italy

alessia.raucci@uniroma1.it

The lysine-specific demethylase 1 (LSD1) catalyses the removal of mono- and dimethyl modifications of Lys4 of histone H3 (H3K4me1/2), which are essential marks of transcriptional activation [1]. LSD1 has been shown to play a central role in the insurgence of solid and blood cancers. In particular, it is highly expressed in acute myeloid leukemia (AML), where LSD1 is crucial for the maintenance of cancer cell stemness, inhibition of cell differentiation, and prevention of apoptosis [2]. Similarly to LSD1, the protein arginine methyltransferase 5 (PRMT5), a methyltransferase that catalyses the symmetric dimethylation of arginine residues [3], acts as an oncoprotein in AML. Indeed, PRMT5 activity was shown to support AML growth *in vitro* and *in vivo* [4]. Given the involvement of both LSD1 and PRMT5 in AML, the simultaneous inhibition of these enzymes may represent a successful approach to treating this malignancy. Notably, we have identified a synergistic interaction between a LSD1 inhibitor and a PRMT5 inhibitor in multiple AML cell lines. The two inhibitors combined promote AML differentiation and eventually growth inhibition and apoptosis. To leverage on this synthetic lethal interaction, we developed a series of dual-targeting LSD1/PRMT5 inhibitors that could inhibit both enzymes *in vitro* in the submicromolar to nanomolar range, while being selective over PRMT1 and PRMT7. Among the prepared compounds, two of them impaired leukemic cell viability with higher potency compared to single-target inhibitors and induced apoptosis and myeloid differentiation. In addition, we were able to solve the X-ray co-crystal structure of one of the designed inhibitors with LSD1, thus elucidating its binding mode and providing a structural basis for the rational design of further inhibitors.

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PC18

UNVEALING NOVEL NITROGEN-BASED COMPOUNDS AS SARS-CoV-2 RNA-DEPENDENT RNA POLYMERASE INHIBITORS.

Ruggieri, G.;[a b] Madia, V. N.;[a] Patacchini, E.;[a] Ialongo, D.;[a] Messore, A.;[a] Albano, A.[a]; Arpacioglu, M.;[a] Malune, P.[c] Esposito, F.;[c] Scipione, L.;[a] Tramontano, E.;[c] Di Santo, R.;[a] and Costi, R.[a]

[a] *Department of Drug Chemistry and Technologies, Pasteur Institute - Cenci Bolognetti Foundation, Sapienza University, 00185 Rome, Italy;*
[b] *Department of Public Health, Experimental Medicine and Forensic, University of Pavia, 27100 Pavia, Italy;*
[c] *Department of Life and Environmental Sciences, University of Cagliari, 09042, Monserrato (CA) Italy.*

PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, 27100, Italy
giuseppe.ruggieri01@universitadipavia.it

For the fifth consecutive year since 2019, the global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections persists, resulting in a growing number of coronavirus disease 2019 (COVID-19) cases worldwide. The primary objective for scientists worldwide is to identify at least one potent antiviral agent capable of effectively halting or disrupting SARS-CoV-2 transmission, cellular entry, replication, and pathogenicity, in the ongoing battle against the resilient COVID-19 disease. However, the arsenal of agents primarily addressing the anti-replication requirements remains limited. Notably, compounds featuring nitrogen-based heterocyclic aromatic cores in their structures, such as nucleoside-like compounds (nucleoside analogs), oxadiazoles, thiadiazoles, triazoles, quinolines, isoquinolines, and certain polyphenolics, have shown significant promise as SARS-CoV-2 inhibitors.[1] Among these, the FDA-approved medications molnupiravir and nirmatrelvir stand out. RNA-dependent RNA-polymerase, which has no counterpart in human cells, is an excellent target for drug development.[2]
Small molecules targeting RdRp are suitable for different motives:

- RdRps inhibitors holds the potential of activity against various viruses because the target is conserved and unlike protease inhibitors, exhibit broader activity because not all viruses encode a protease;
- Polymerases present a high natural genetic barrier to drug resistance due to their conserved nature, making polymerase inhibitors a promising option for developing broad-spectrum antiviral agents;
- The current therapy against RdRp lacks small molecules because most of the inhibitors are nucleos(t)ide analogues administrated by iv (*e.g.* Remdesivir) or orally even if they showed several side effect (*e.g.* Molnupiravir).

For this reason, we decided to invest our effort for the identification of novel nitrogen-based small molecules targeting. In this regard, we conducted an extensive screening of previously synthesized in-house antiviral compounds. Our focus was primarily on non-nucleos(t)idic compounds featuring a central nitrogen core. Following this preliminary screening, several compounds underwent testing using the RdRp SARS-CoV-2 Primer-elongation assay on PAGE. Among those exhibiting residual enzymatic activity below 50% (up to 30 μM) post-treatment, the SARS-CoV-2 RdRp $IC_{50} \pm SD$ (μM) was determined. Remarkably, some compounds displayed noteworthy in vitro activity, with IC50 values similar to the control drug, Simeprevir.
Concurrently, we designed and synthesized novel derivatives that are presently undergoing biological evaluation. In this way we can elucidate structure-activity relationships (SARs) and obtaining a novel series of nitrogen-based compounds to test against other viral RdRps. The findings from this screening will be presented and discussed.

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PC19

A Novel Carbonic Anhydrase and Wnt/β-Catenin Signaling Pathway Dual-Targeting Inhibitor with Potent Activity against Multidrug Resistant Cancer Cells

M. Santelli,[a] D. Masci,[a] M. Puxeddu,[b] A. Urbani,[a] E. Hamel,[c] C.T. Supuran,[d] R. Silvestri,[b] G. La Regina[b]

[a]Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Catholic University of theSacred Heart, Largo F. Vito 1, 00168 Rome, Italy; [b]Laboratory Affiliated with the Institute Pasteur Italy Cenci Bolognetti Foundation, Department of Drug Chemistry andTechnologies, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [c]Frederick National Laboratory for Cancer Research, National Cancer Institute, National Institutes of Health,Frederick, Maryland 21702, United States; [d]Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, ViaUgo Schiff 6, I-50019 Sesto Fiorentino, Florence, Italy.

martina.santelli01@icatt.it

Carbonic anhydrases (CAs) are ubiquitous enzymes that catalyze the reversible hydration of carbon dioxide to produce monohydrogen carbonate and H^+ ions. The CA isoforms play key roles in many physiological processes: signal transduction, cell differentiation and proliferation, and onco-genesis[1]. The project starts from the relevance of the sulfonamide group as inhibitor of several isoforms of carbonic anhydrase. We decided to start from different derivatives, active as tubulin polymerization inhibitors, introducing a sulfonamide group, to selectively inhibit CA IX and CA XII isoforms, which are triggered by hypoxia-inducible factor 1 in manytypes of cancer. Novel pyrrole and indole derivatives have been synthesized as human carbonic anhydrase (hCA) inhibitors. The presence of both N1-(4-sulfonamidophenyl) and 3-(3,4,5-trimethoxyphenyl) substituents was essential for strong hCA inhibitors. Notably, compound 15, the most potent hCA XII inhibitor (Ki = 6.8 nM), demonstrated a dual target inhibition by effectively suppressing the Wnt/β-catenin signaling pathway as well, which is found to be dysregulated in some solid tumors, its target genes MYC, Fgf20, and Sall4 and exhibited the typical markers of apoptosis[2]. Docking experiments were performed to gain insights into the molecular details of the binding modes of the reported compounds. Several compounds exhibited promising activity against different tumor cell lines, including colorectal cancer and triple-negative breast cancer, highlighting their potential as therapeutic agents.

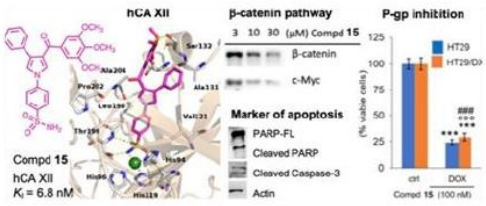

Figure 1: Compound 15 showed strong inhibition of viability in a panel of cancer cells.

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PC20

DESIGN OF CINNAMIC ACID DERIVATIVES CONTAINING A PIPERIDINE OR PIPERAZINE MOIETY AS PROMISING AGENTS FOR NEURODEGENERATIVE DISORDERS

Konstantina Sdougkou (1), Georgios Papagiouvannis (2), Iokasti Lazidou (1), Dionisia Papagiannopoulou(1), Eleni Rekka (1)

1) Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
2) Department of Pharmacy, School of Health Sciences, Frederick University, Nicosia, 1036, Cyprus

Alzheimer's disease (AD) is a chronic neurodegenerative disease that affects millions of people worldwide and the number is expected to increase due to the rise of life expectancy and the aging population. There is no effective therapy so far available, although some acetylcholinesterase (AChE) inhibitors such as donepezil and N-methyl-d-aspartate (NMDA) receptor antagonists e.g. memantine are in clinical use for symptomatic treatment. However, there are many pathobiochemical changes in the demented brain, which could be used as a basis for designing better agents against AD. Such changes are dysregulation of neurotrophic factors, inflammation and oxidative stress. Moreover, GABAergic neurotransmission is affected, contributing to behavioral dysfunction in AD [1]. Therefore, we have designed, synthesized and studied some novel compounds, which are expected to possess the desired properties. Carboxylic acids (e.g. sinapic acid, ferulic acid) which were esterified or amidated with substituted piperidine or piperazine groups, are expected to combine antioxidant and acetylcholinesterase antagonistic activity. Firstly, a docking simulation of compounds on human AChE active site was studied in a preliminary assessment in order to explore their mechanism of action. The estimated binding energy of examined compounds in the active site of AChE was compared with that of donepezil.

All compounds were synthesized applying standard techniques, purified by flash column chromatography and identified spectroscopically. The synthesized compounds were tested for antioxidant activity, activity against protein glycation induced by reducing sugars (fructose) and their inhibitory capacity against AChE as well as the effect of selected compounds on acute inflammation. It was found that compounds bearing a phenolic hydroxyl moiety were able to inhibit lipid peroxidation.

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PC21

Searching for allosteric and biased ligands of the mi opioid receptor (MOP)

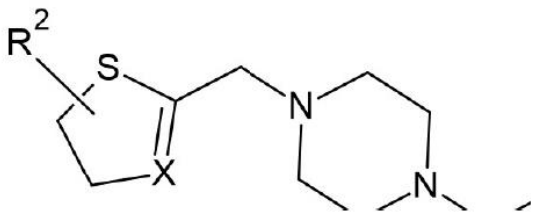
Jagoda Sokołowska-Duda,[a] Dariusz Matosiuk[a]

[a] Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modelling Lab, Medical University of Lublin, 4A Chodźki Str., PL20093 Lublin, Poland

jagoda.sokolowska.55@gmail.com

Opioids represent a class of chemical compounds characterized by their ability to bind to opioid receptors present in the brain and other tissues of the body. Both natural and synthetic opioids exhibit a propensity to interact with these receptors, leading to various biological effects, including analgesia, sedation, and euphoria. As a result, they find application in the therapy of pain of various etiologies.

The previous studies that were performed made it possible to discover new groups of compounds capable of modulating the activity of morphine. They potentiated the activity of morphine in subthreshold doses and DAMGO also in subthreshold (inactive) doses. Morphine and DAMGO in these experiments showed effects as if given in much stronger doses (10-50 times stronger) [1]. Computer models of the receptors and virtual docking of ligands have uncovered allosteric binding sites in the OP3 receptor [2] and revealed the mechanism of receptor activation, in which helixes VI and VII are significantly involved [3].



Using computer models, we searched the Enamine compound database, confirming potential activity to the allosteric site for many derivatives, from which we selected the 20 most active. Several of these contained the piperazine system as the basic heterocyclic system, and these compounds became the focus of our research.

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PC22

Amides derived from 3,5-di-*tert*-butyl-4-hydroxycinnamic acid as potential anti-inflammatory drugs: Design, synthesis and assessment of COX-2 inhibition and selectivity

João Sousa Janela[a], Mariana Lucas[b], Marisa Freitas[b], Elisiário Tavares da Silva[a], Eduarda Fernandes[b], Fernanda Roleira[a]

[a] CERES, University of Coimbra, Faculty of Pharmacy, 3000-548 Coimbra, Portugal
[b] LAQV, REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
joaojanela@ff.uc.pt

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammatory diseases by inhibiting the enzyme cyclooxygenase (COX), which has two main isoforms: COX-1, which is expressed constitutively and is primarily involved in maintaining homeostatic functions, and COX-2, which is induced by pro-inflammatory stimuli and plays a significant role in promoting inflammation. First-generation NSAIDs, lacking COX selectivity studies, caused gastric ulcers and bleeding. Selective COX-2 inhibitors were developed to mitigate this but increased cardiovascular risks emerged. Previously, our team found that 3,5-di-*tert*-butyl-4-hydroxycinnamic acid and its respective hexylamide and diethylamide were selective COX-2 inhibitors based on a prostaglandin E_2 (PGE_2) quantification assay in human blood [1]. Our group has now synthesized new amides from the same hydroxycinnamic acid, by incorporating different *N*-alkyl and *N*-aryl groups (Figure 1). These compounds were evaluated for their inhibitory effects on COX-1 and COX-2, using an *in vitro* fluorometric screening assay. Furthermore, the selectivity of the compounds for the COX-2 isoform was determined. The PGE_2 quantification assay was also conducted under COX-2 stimulating conditions, to determine if the activity of the compounds observed in the *in vitro* model translates to a biological sample. Among the tested compounds, we have found several selective COX-2 inhibitors with IC_{50} values as low as 1.4 µM (in isolated human COX-2) or 8.2 µM (in human blood). These results highlight the potential of the newly synthesized amides as promising candidates for selective COX-2 inhibition and warrant further investigation.

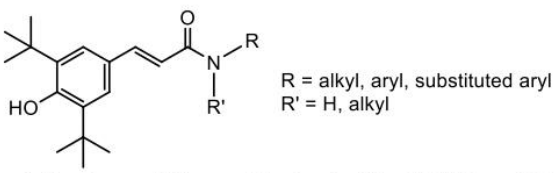

Figure 1: General structure of the amides tested for COX-1 and COX-2 inhibition.

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PC23

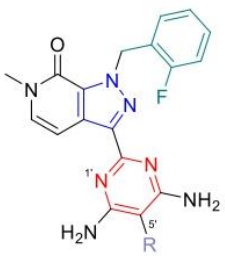
New pyrazolo[3,4-*c*]pyridone derivatives with soluble guanylyl cyclase agonistic activity

Aggeliki Christopoulou*, Dionisis-Panagiotis Kintos*, Konstantinos Toufas*, Konstantinos Laimos-Geranios*, Antonios Alatzakis**, Christina Kalogirou**, Sotirios Nikolaropoulos*, Stavros Topouzis**, Manolis Fousteris*

**Laboratory of Medicinal Chemistry, Department of Pharmacy, University of Patras, Patras GR-26500, Greece; E-mail: manolisf@upatras.gr*
***Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras GR-26500, Greece.*

Soluble guanylyl cyclase (sGC) is a heterodimeric enzyme involved in the NO/sGC/cGMP signaling pathway. Nitric oxide (NO) activates sGC by binding to the ferrous heme-moiety in the H-NOX (Heme-NO Oxygen) domain of the β1 subunit. This interaction triggers conformational changes facilitating enzyme activation and cyclic monophosphoric guanosine (cGMP) formation.[1] Activation of sGC regulates a plethora of cellular and physiological responses such as vasodilation, smooth muscle proliferation, leukocyte recruitment, and platelet aggregation.[2] Dysregulation of this pathway can derive from diminished NO synthesis, oxidative stress, and increased cGMP degradation, resulting in a variety of pathological conditions with endothelial dysfunction being the leading one.[2] Given the limitations of the available treatments (NO donors and PDE inhibitors), a new class of therapeutic agents, which directly enhance the sGC enzymatic activity, the sGC stimulators, has been developed.[3] These molecules bind allosterically to the enzyme and have a dual mode of action by stimulating the native enzyme directly without the presence of NO and exhibiting a synergistic effect with endogenous NO.[3,4]

Recently, we have identified new pyrazolo[3,4-*c*]pyridin-7(6*H*)-ones (**I**) as sGC stimulators, with vasoprotective and anti-inflammatory activities.[5] These molecules incorporate crucial structural features of known sGC stimulators while the pyrazolopyridone skeleton may be implicated in efficient binding interactions with the enzyme. In an effort to improve the sGC agonistic activity of these compounds, we perform a structure-activity relationship study by modifying appropriately specific moieties around the pyrazolopyridone scaffold. In particular, we designed and synthesized new derivatives by introducing various bioisosteric groups at the C5' position of the pyrimidine ring. Herein, the design, synthesis and the sGC stimulating activity of these new derivatives will be presented.



(I)
R = various *N*-H and *N*-Me containing groups

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PC24

Synthesis and Biological Evaluation of Macrocyclic AURKA Inhibitors

Anouk Van Hauwermeiren[a], Simon Krols[a], Hanne Van De Putte[a], Muhammad Rishifi[b], Fien Martens[b], Kaat Durinck[b], Serge Van Calenbergh[a]

a. Laboratory of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Belgium
b. Department of Biomolecular Medicine, Faculty of Medicine & Health Sciences, Ghent University, Belgium

Ottergemsesteenweg 480, Ghent 9000, Belgium
Anouk.VanHauwermeiren@UGent.be

Aurora kinases A (AURKA) and B (AURKB) are oncogenes implicated in various types of cancer, with AURKA playing a critical role in promoting cancer cell growth. (1) Although numerous clinical trials have tested Aurora kinase inhibitors for cancer therapy, the use of pan-Aurora kinase inhibitors is limited due to toxicity from AURKB and AURKC inhibition. (2) Recent data suggest that macrocyclization can significantly improve a compound's biological and physicochemical properties by locking it in the bioactive conformation enhancing selectivity, even among closely related targets, and reducing the entropic cost of binding, thereby increasing potency. This approach could explore a new chemical space for the discovery of novel kinase inhibitors. (3–7)

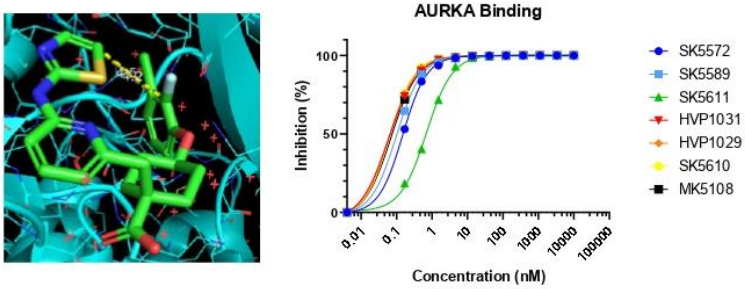

Figure 1. The image on the left shows a co-crystal structure of MK-5108 bound to AURKA. The graph on the right shows the binding curves of the synthesized macrocyclic compounds compared to MK-5108.

A co-crystal structure of MK-5108, a selective AURKA inhibitor, bound to AURKA suggests potential for macrocyclization between the ortho/meta positions of the phenyl ring and the thiazole moiety. Based on this, macrocyclic AURKA inhibitors were synthesized and evaluated for their binding affinity to AURKA, and selectivity versus AURKB. The macrocyclic compounds showed similar or slightly improved affinity for AURKA compared to MK-5108. However, in vitro cell-based assays revealed no significant growth inhibition in NGP cells (GI50 > 1000 nM), likely due to permeability issues or inadequate target engagement. To address this, a nanoBRET study in both permeabilized and intact cells is planned to identify the specific issues. Furthermore, a new series of macrocyclic compounds is being synthesized with the aim of improving their physicochemical properties.

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Novel 5-HT$_{2A}$ receptor ligands retrieved from a virtual screening campaign

Agata Zięba[a*], Damian Bartuzi[a,b], Piotr Stępnicki[a], Dariusz Matosiuk[a], Tomasz M. Wróbel[a], Tuomo Laitinen[c], Marián Castro[d,e], Ewa Kędzierska[f], Tadeusz Karcz[g], and Agnieszka A. Kaczor[a,c]

[a] Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modelling Lab, Medical University of Lublin, Lublin, Poland [b] Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden [c] School of Pharmacy, University of Eastern Finland, Kuopio, Finland [d] Department of Pharmacology, Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases, Santiago de Compostela, Spain [e] Instituto de Investigación Sanitaria de Santiago de Compostela, Santiago de Compostela, Spain [f] Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland [g] Department of Technology and Biotechnology of Drugs, Jagiellonian University, Medical College, Kraków, Poland

How are you? This is a question we hear nearly every day. Regardless of how sophisticated our answers may be, mood is regulated by numerous neurochemical mechanisms, with serotonin playing a particularly important role. Dysregulation of serotonin neurotransmission is commonly identified in many mental health disorders, including mood disorders. Notably, these conditions have become a significant global burden, with an estimated 970 million people affected worldwide—a number expected to rise in the coming years. Many pharmaceutical interventions aiming to help patients suffering from mental health disorders target the serotonergic system, comprising mostly GPCRs, with the 5-HT$_{2A}$ receptor as one of the most widely studied components. Despite the wide range of agents available, many of them result in side effects or delayed onset of action, diminishing the safety and effectiveness of the treatment.

Encouraged by the plethora of pharmaceutical applications offered by ligands targeting the 5-HT$_{2A}$ receptor, we constructed a virtual screening campaign to select novel potential compounds interacting with this molecular target. Subsequent *in vitro* assays confirmed the antagonist activity of 6 compounds. Molecular dynamics simulations offered insight into the binding mode for these ligands, and attempted to explain the mechanism in which they interacted with the main molecular target. Subsequently, these molecules were evaluated for a binding affinity at 5-HT$_{1A}$, 5-HT$_7$, D$_2$ receptors. That is, targets characterized by a high degree of similarity to the 5-HT$_{2A}$ receptor and widely implicated in the development of CNS pathologies[1]. Equipped with this preliminary data, we focused on the determination of the basic ADME-TOX properties of these compounds in *in vitro* assays, and confirmation of their antidepressant properties in *in vivo* studies. These experiments served as a starting point in the campaign aimed at discovering novel ligands potentially valuable for the treatment of mental disorders.

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[1] A. Zięba, D. Bartuzi, P. Stępnicki, D. Matosiuk, T. M. Wróbel, T. Laitinen, M. Castro, A. A. Kaczor, *ChemMedChem* https://doi.org/10.1002/cmdc.202400080.