Euro PhD Network

P Ehrlich MedChem

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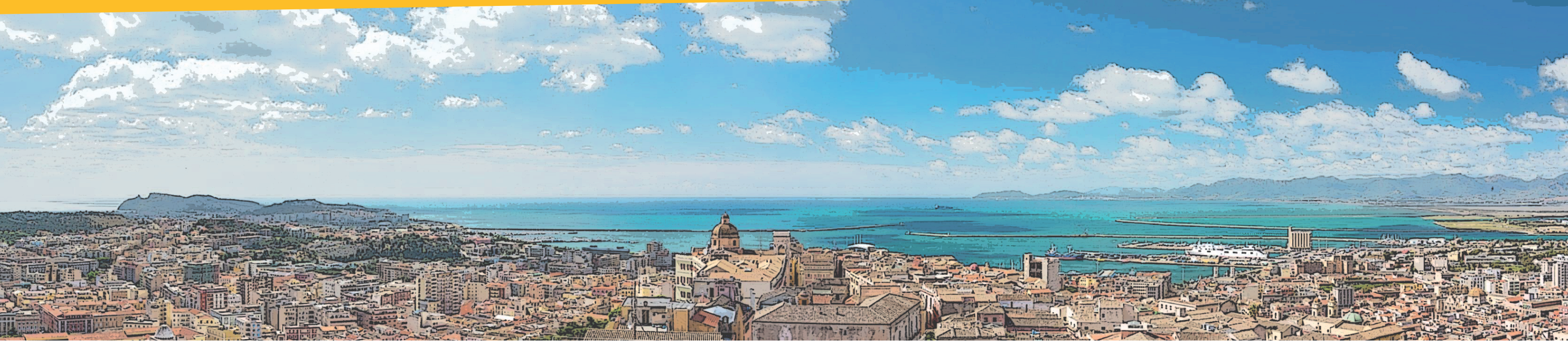

Book of Abstracts

XV Paul Ehrlich Euro-PhD Network Meeting 2026

20th-22nd May, Flamingo Hotel
Santa Margherita di Pula, Cagliari, Italy

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WELCOME PREFACE

We are pleased to announce the upcoming International Paul Ehrlich MedChem 2026 conference (#PE2026) – May 20th – 22nd, 2026 – at the Hotel Flamingo Resort in Santa Margherita di Pula (CA), Italy, organized by the "CADD, Synthesis and Extraction of Bioactive Compounds MedChem" research group with the support of the University of Cagliari (UNICA).

#PE2026 is designed to be a significant showcase for young PhD students and researchers in the field of medicinal chemistry. It promotes knowledge exchange by sharing research results and involving production activities. The meeting will foster interaction and the exchange of the latest scientific discoveries within the Medicinal Chemistry community in a lively, international setting.

I hope that during this event, we will stay engaged, proactive, and support each other in shaping the future of the Paul Ehrlich MedChem Euro-PhD Network.

We look forward to seeing you in Cagliari.

Prof Elias Maccioni

Meeting Chairman and Scientific Coordinator

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SCIENTIFIC COMMITTEE

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(University of Porto)
(*PE Network Coordinator and Vice-Chair of MedChem 2026*)

Elias Maccioni
(University of Cagliari)
(*Meeting Chairman and Scientific Coordinator of MedChem 2026*)

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(University of Nantes)
(*Organizer 2025 meeting*)

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(Ghent University)
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(University of Cagliari)

Antonio Lupia
(University of Cagliari)

ORGANIZING COMMITTEE

Synthetic & CADD Labs

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Laura Demuru

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Rita Meleddu

Alessia Onali

Erica Sanna

#PE2026 SPEAKERS

Plenary Lecture

Prof. Dr. Danijel Kikelj

Danijel Kikelj is Professor Emeritus of medicinal chemistry at the Faculty of Pharmacy, University of Ljubljana, Slovenia. He graduated in pharmacy from the University of Ljubljana in 1978, where he also earned a Master of Science degree in 1983. He obtained his doctorate (Dr. rer. nat.) in heterocyclic pharmaceutical chemistry from the University of Heidelberg, Germany, in 1988 under the mentorship of Professor Richard Neidlein. From 1991 to 1992, he served as a C-3 professor of pharmaceutical chemistry at the University of Heidelberg. From 2000 until his retirement in 2022, he was a full professor of medicinal chemistry and head of the research programme in Medicinal Chemistry at the Faculty of Pharmacy of the University of Ljubljana. The main areas of his research have been medicinal chemistry, peptidomimetics, muramyl dipeptide-based immunomodulatory compounds, structure-based design of dual antithrombotic compounds, inhibitors of Mur enzymes involved in peptidoglycan biosynthesis, pharmaceutical leads based on marine bioactive compounds, and, most recently, the discovery of inhibitors of bacterial topoisomerases with activity against Gram-negative bacteria.

He was one of the founders of the Paul Ehrlich MedChem Euro-PhD Network, organized the 2nd Network meeting at the University of Ljubljana in 2012, and served as the Network's coordinator from 2012 to 2015.

Invited Lecture

Prof. Ana Martinez

Ana Martinez, Research Professor at CIB-CSIC, is a leading figure in medicinal chemistry with over 35 years of experience in translational drug design. Her career is defined by an exceptional ability to bridge the gap between fundamental research and clinical application, primarily focusing on neurodegenerative and infectious diseases. Since 2010, she has dedicated her research to drug discovery for ALS, successfully advancing protein kinase inhibitors to the clinical stage, and founding the spin-off MOLEFY Pharma to support their development. Her prolific scientific output includes more than 350 manuscripts in peer-reviewed journals, 40 patent families, and half a dozen edited books, accumulating over 12,000 citations. These contributions have earned prestigious achievements, including awards from the Spanish Patent and Trademark Office (OEPM), the Madrid Regional Government, and the Royal Academy of Pharmacy (RANF). In 2022, she was recognized with the National Research Award "Juan de la Cierva" in Technology Transfer and the Margarita Salas Medal for excellence in scientific supervision.

LinkedIn

Invited Lecture

Dr. Ed J. Griffen

Ed J. Griffen earned his PhD in synthetic organic chemistry from Imperial College London, followed by postdoctoral research at the University of Waterloo, Canada. He subsequently joined AstraZeneca as a medicinal chemist, where he progressed to the role of Principal Scientist. In 2012, he co-founded MedChemica Ltd. He is an inventor of 17 patents and has authored more than 30 scientific articles as well as a textbook. He has contributed to four drug discovery projects that have progressed to clinical development, including capivasertib. In March 2020, he joined the COVID Moonshot initiative as Design Team Leader, contributing to the development of two SARS-CoV-2 inhibitors that advanced to pre-clinical development. Since 2022, he has been Head of Lead Optimisation at the ASAP Pandemic Preparedness U19 AViDD Centre, where he is currently working on treatments for flaviviral infections, including dengue and Zika viruses. He is also a co-Principal Investigator in the OpenBind Consortium, which aims to build large-scale datasets for the prediction of binding affinity.

LinkedIn

Keynote Lecture

Prof. Alessandra Montalbano

We are pleased to announce that Prof. Alessandra Montalbano will deliver a keynote lecture entitled "Thiazole-Based Derivatives as a Versatile Scaffold in the Inhibition of Respiratory Viruses." The lecture will present recent advances in the development of thiazole-based derivatives as promising antiviral agents, highlighting their potential in the inhibition of SARS-CoV-2 Nsp13 helicase and Influenza A/H1N1 viral entry, paving the way for the design of novel broad-spectrum antiviral candidates.

SCIENTIFIC PROGRAMME

Wednesday, May 20

From 12:00 PM- Registration

***02:45 PM- Welcome & Opening PE2026* – SALA NAUTILUS**
Fernanda Borges and Elias Maccioni

03:00 PM– Plenary Lecture
Prof. Dr. Danijel Kikelj
"Paul Ehrlich MedChem Euro-PhD Network: A Permanent Inspiration"

***Oral Communications* (10 + 3 min Q&A)**

Chairs:
Claudia Sissi and Jarmila Vinsova

04:00 PM
***OC1* – Ewout Van de Velde**
Ghent University
Generalized parallel synthesis and anti-trypanosomal evaluation of a chemically diverse 6-functionalized C-nucleoside compound library
Author Information & E-Abstract

04:15 PM
***OC2* – Ana Carolina Mazzochi**
Universidade de Santiago de Compostela
Anti-Trypanosoma cruzi Potential of New Pyrazole-Imidazoline Derivatives
Author Information & E-Abstract

04:30 PM
***OC3* – Sara Damiani**
University of Ljubljana
Synthesis and Investigation of Fluorinated Scaffolds Exhibiting Through-Space Scalar ^{19}F –^{19}F NMR Coupling
Author Information & E-Abstract

04:45 PM
***OC4* – João Sousa Janela**
Universidade de Coimbra
New O-Prenylated Caffeic Acid Amides with Selective Toxicity Against Trypanosoma cruzi Amastigotes. Design, Synthesis and Structure-Activity Relationships
Author Information & E-Abstract

Coffee Break

Chairs:
Fernanda Roleira and Giulio Vistoli

05:30 PM
***OC5* – Laura Demuru**
University of Cagliari
Identification, Design, and Optimization of New Allosteric Inhibitors Against ZIKV and WNV NS2B-NS3^{pro}
Author Information & E-Abstract

05:45 PM
***OC6* – Leonardo Lemos**
University of Santiago de Compostela
Synthesis, characterization and evaluation of anti-T. cruzi activity of 1-arylpyrazole-4-carboximade-N-propylimidazoles
Author Information & E-Abstract

06:00 PM
***OC7* – Federico Faloci**
University of Padua
Expanding the Therapeutic Potential of Purine C-Nucleosides from Chemical Synthesis to Oligonucleotide Incorporation
Author Information & E-Abstract

06:15 PM – Flash Poster communications Author Information & E-Abstract

07:30 PM – Welcome Cocktail & Dinner

Thursday, May 21

Chairs:
Maria Laura Bolognesi and Chris Meier

08:30 AM- Invited Lecture
Prof. Ana Martinez
“TDP-43 Modulation in ALS: From Bench to Bedside”

09:15 AM
***OC8* – Bárbara Albuquerque**
University of Porto
Evaluation of mitochondrial toxicity induced by a novel COMT inhibitor
Author Information & E-Abstract

09:30 AM
***OC9* – Ayesha Asim**
Medical University of Lublin
Dual-Target Drug Discovery in Alzheimer’s Disease Using Machine Learning guided approach integrated with structure based virtual screening
Author Information & E-Abstract

09:45 AM
***OC10* – Claudia Machado**
University of Porto
Nanocarrier-Based Co-Delivery of Edaravone and Riluzole for Improved ALS Treatment Strategies
Author Information & E-Abstract

Coffee Break & Poster Session

Chairs:
Oriana Tabarrini and Serge Van Calenbergh

11:00 AM

***OC11* – Alice Fossati**

University of Pavia

Identification of Natural Tyrosinase Inhibitors as potential therapy for Neurodegenerative Diseases

Author Information & E-Abstract

11:15 AM

***OC12* – Mariana Castelôa**

University of Porto

Chasing NOX4: From VAS2870 to a Potent, Selective, Covalent Inhibitor

Author Information & E-Abstract

11:30 AM

***OC13* – Manuel Novás**

Universidade de Santiago de Compostela

Pharmacomodulation of 2-Amidobenzothiazoles for the Development of Neuroprotective Compounds: A Phenotypic Study in an Alzheimer's Disease Model

Author Information & E-Abstract

11:45 AM

***OC14* – Carla Lima**

University of Porto

Targeting Mitochondrial Iron Dysregulation in Friedreich's Ataxia Using Novel Chelators

Author Information & E-Abstract

12:00 PM

***OC15* – Vince Bart Cardenas**

Università della Svizzera Italiana

Beyond the 1:1 Ligand-Protein Paradigm: An In Silico Assay for Competitive Ligand Binding

Author Information & E-Abstract

01:00 PM – Lunch and Relax Time (Before Lunch Group Photo)

02:30 PM– PE Board Meeting

Chairs:
Maria Joao Matos and Stefano Alcaro

03:15 PM – Invited Lecture
Dr. Ed J. Griffen
"The discovery of new orally active direct-acting antivirals for lethal Coronaviruses"

04:00 PM
***OC16* – Caterina Deruvo**
Università degli Studi di Bari "A. Moro"
Investigation of novel guaiacol-bearing molecules as multi-targeted directed ligands (MTDLs) against Alzheimer's disease-related targets
Author Information & E-Abstract

04:15 PM
***OC17* – Fabiana Lo Mascolo**
University of Palermo
Prodrug strategies to improve the drug-like properties of fragment-derived Keap1-Nrf2 Inhibitors
Author Information & E-Abstract

04:30 PM
***OC18* – Ruben Tack**
Ghent University
Towards Closed-Loop Spatiotemporal Treatment of Drug-Resistant Epilepsy With Photopharmacology
Author Information & E-Abstract

Coffee Break & Poster Session

Chairs:
Maria Paola Costi and Dariusz Matosiuk

05:15 PM
***OC19* – Nicolò Dal Ponte**
University of Padova
Targeting the vimentin-G4 repeats axis to disrupt EMT and cancer cell invasion
Author Information & E-Abstract

05:30 PM
***OC20* – Marta Duran Martinez**
Universidad de Alcalá
New PROTAC approaches targeting the pseudokinase ILK
Author Information & E-Abstract

05:45 PM
***OC21* – Marzia Fois**
University of Ljubljana, Faculty of Pharmacy, Slovenia
Targeting Mitochondrial KV1.3 in Cancer: Development of Mitochondria-Directed Inhibitors
Author Information & E-Abstract

06:00 PM
***OC22* – Angela Santo**
University of Bari
Integrating Medicinal Chemistry and Bioinformatics to Advance Knowledge on P2X7R in Neuroinflammation and Tumor Immunity
Author Information & E-Abstract

06:15 PM
***OC23* – Francesco Brogi**
University of Perugia
Chemical Probes for Mono-ADP-Ribosylating enzymes (mono-ARTs) inhibition and degradation
Author Information & E-Abstract

08:00 PM – Gala Dinner at the Wild Duck Restaurant

Friday, May 22

Chairs:
Athina Geronikaki and Cosimo Damiano Altomare

09:00 AM- Keynote **(20 min + Q&A)**
Prof. Alessandra Montalbano
University of Palermo, Italy
“Thiazole-Based Derivatives as a Versatile Scaffold in the Inhibition of Respiratory Viruses”

09:30 AM
***OC24* – Martina Garbagnoli**
University of Pavia
BRAC ligands: novel small molecules targeting the HuR-RNA complexes with a broad-spectrum anticancer activity
Author Information & E-Abstract

09:45 AM
***OC25* – Emiliano Paradiso**
University of Ljubljana, Faculty of Pharmacy, Slovenia
Conjugated NOD1/PRR Agonists Reveal Distinct In Vitro and In Vivo Immune Signatures
Author Information & E-Abstract

10:00 AM
***OC26* – Emanuele Fabbrizi**
Sapienza University of Rome, Italy
Targeting m6A Epitranscriptomic Machinery: Design and Biological Characterization of Potent Quinazoline-Derived METTL3–METTL14 Inhibitors
Author Information & E-Abstract

10:15 AM
***OC27* – Anouk Van Hauwermeiren**
Ghent University
Macrocyclization as a strategy for optimizing PROTACs targeting Aurora Kinase A
Author Information & E-Abstract

Coffee Break

Chairs:
Fernanda Borges, Elias Maccioni, and Antonio Lupia

PE-Alumni networking presentation & PE-Medchem Euro PhD Label Communications
(15 +3 min Q&A)

11:10 AM
***PE Award 1* – Laura Marquez-Cantudo**
Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo-CEU, Spain
Computer-aided drug design and evaluation of enzyme inhibitors, PROTACs and LYTACs targeting cancer and other diseases
Author Information & E-Abstract

11:30 AM
***PE Award 2* – Giorgio Buttitta**
Sapienza University of Rome, Italy
Liposomes: the gold standard in drug delivery technology
Author Information & E-Abstract

11:50 AM
***PE Award 3* – Adriana Gargano**
Università Magna Graecia di Catanzaro – Italy
Discovery and Characterization of Natural Compounds for Cancer Therapy: A Combined Target-Based and Phenotypic Approach
Author Information & E-Abstract

12:10 PM
***PE Award 4* – Bárbara Nunes**
LEPABE, ALiCE, Faculty of Engineering, University of Porto, Portugal
Quaternary ammonium and phosphonium salts as antimicrobial agents: synthesis, biological evaluation, and mechanistic insights
Author Information & E-Abstract

12:30 PM

***PE Award 5* – Emanuela Marchese**

Università Magna Graecia di Catanzaro – Italy

In Silico Approaches to Uncover the Antioxidant and Therapeutic Properties of Polyphenols

Author Information & E-Abstract

01:00 PM – Lunch

Chairs:

Fernanda Borges, Daniela Secci, and Rafal Latajka

02:30 PM

***PE Award 6* – Yushun Ihor**

Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Ukraine

Synthesis and biological activity of thiazolidine derivatives with a pyrazoline fragment in molecules

Author Information & E-Abstract

02:50 PM

***PE Award 7* – Martina Piga**

University of Ljubljana, Faculty of Pharmacy, Slovenia

Targeting NaV and HV1 channels: discovery of novel small-molecule inhibitors

Author Information & E-Abstract

03:10 PM

***PE Award 8* – Miguel Pinto**

RISE-Health, Faculty of Medicine, University of Porto, Porto, Portugal

Using a nanomedicine-based approach as a second chance for tolcapone

Author Information & E-Abstract

03:30 PM

***PE Award 9* – Lorenzo Raffellini**

University of Pisa, Italy

Targeting autophagy through AMPK: design, synthesis, and biological evaluation of novel allosteric modulators

Author Information & E-Abstract

03:50 PM

***PE Award 10* – Benedetta Salvucci**

National Center Rare Diseases, Istituto Superiore di Sanità, Rome, Italy

Development of Novel Antimalarial Transmission Blocking Agents and Genetic Characterization of Drug Resistance in Mycobacterium abscessus

Author Information & E-Abstract

Awards and Closing Remarks

Fernanda Borges, Elias Maccioni, and the Organizing Committee

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Social Event

LIST OF ABSTRACTS

ORAL COMMUNICATIONS (OCs)

OC1	*Ewout Van de Velde*	*Generalized parallel synthesis and anti-trypanosomal evaluation of a chemically diverse 6-functionalized C-nucleoside compound library*
OC2	*Ana Carolina Mazzochi*	*Anti-Trypanosoma cruzi Potential of New Pyrazole-Imidazoline Derivatives*
OC3	*Sara Damiani*	*Synthesis and Investigation of Fluorinated Scaffolds Exhibiting Through-Space Scalar ^{19}F –^{19}F NMR Coupling*
OC4	*João Sousa Janela*	*New O-Prenylated Caffeic Acid Amides with Selective Toxicity Against Trypanosoma cruzi Amastigotes. Design, Synthesis and Structure-Activity Relationships*
OC5	*Laura Demuru*	*Identification, Design, and Optimization of New Allosteric Inhibitors Against ZIKV and WNV NS2B-NS3^{pro}*
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OC21	*Marzia Fois*	*Targeting Mitochondrial KV1.3 in Cancer: Development of Mitochondria-Directed Inhibitors*
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OC24	*Martina Garbagnoli*	*BRAC ligands: novel small molecules targeting the HuR-RNA complexes with a broad-spectrum anticancer activity*
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OC26	*Emanuele Fabbrizi*	*Targeting m6A Epitranscriptomic Machinery: Design and Biological Characterization of Potent Quinazoline-Derived METTL3–METTL14 Inhibitors*
OC27	*Anouk Van Hauwermeiren*	*Macrocyclization as a strategy for optimizing PROTACs targeting Aurora Kinase A*

Generalized parallel synthesis and anti-trypanosomal evaluation of a chemically diverse 6-functionalized C-nucleoside compound library

Ewout Van de Velde[1], Lisa Marriottini[1], Anouk Van Hauwermeiren[1], Guy Caljon[2], Maria de Nazare Correira Soeiro[3], Serge Van Calenbergh[1]

1. Ghent University, Faculty of Pharmaceutical Sciences, Laboratory for Medicinal Chemistry;
2. University of Antwerp, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Laboratory of Microbiology, Parasitology and Hygiene
3. Laboratório de Biologia Celular do Instituto Oswaldo Cruz, Fiocruz, Avenida Brasil 4365, ManguinhosRio de Janeiro, Brazil

ewout.vandevelde@ugent.be

Keywords: *late-stage functionalization, nucleoside analogues, trypanosomatid infections*

Neglected Tropical Diseases (NTDs) caused by vector-borne protozoan parasites—including African sleeping sickness, Chagas disease, and leishmaniasis—disproportionately affect impoverished populations in tropical and subtropical regions. Current treatments suffer from toxicity, complex administration, and emerging resistance, underscoring the urgent need for safer, selective antiparasitic agents.

C-Nucleosides, defined by a carbon–carbon glycosidic bond, resist enzymatic cleavage by hydrolases and phosphorylases and allow systematic variation in ring composition to generate novel pharmacophores. The demonstrated in vivo activity of 9-deazainosine and formycin B against Trypanosoma and Leishmania highlights their therapeutic potential, yet efficient access to structurally diverse C-nucleoside libraries remains limited. Here we report a late-stage diversification platform, enabled by a 3,5-bis(trifluoromethyl)phenoxy (BTFPO) handle, that allows rapid assembly of C-nucleosides bearing three distinct purine-like scaffolds and a wide range of substituents from stable, versatile intermediates. This approach consistently delivers potent, selective anti-trypanosomatid agents, as demonstrated by the in vivo efficacy and safety of lead compound 58 in two distinct mouse models.

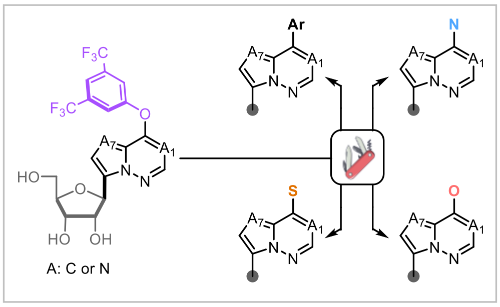

Figure 1. Late-stage functionalization of C-nucleoside analogues by use of BTFPO

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Anti-*Trypanosoma cruzi* Potential of New Pyrazole-Imidazoline Derivatives

Mazzochi Ana Carolina[1], Santos Maurício S.[1], Fernandes Pedro H. M.[1], Oliveira Edinaldo C.[2], Lara Leonardo S.[2], Orlando Lorraine M. Rocha[2], Lanera Sarah C.[2], Souza Thamyris P.[2], Figueiredo Nathalia S.[2], Paes Vitoria Barbosa[2] and Pereira Miriam C. de Souza[2]

1. Laboratório de Síntese de Sistemas Heterocíclicos (LaSSH), Instituto de Física e Química (IFQ), Universidade Federal de Itajubá, Itajubá, 37500-903, Brazil;
2. Laboratório de Ultraestrutura Celular, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, 21040-900, Brazil;

caromazzochi@gmail.com

Keywords: *Trypanosoma cruzi, pyrazole-imidazoline, Chagas disease, chemotherapy,* and *3D culture model*

Chagas disease (CD) is a major public health concern. The prevalence of the disease has been documented in 21 Latin America countries, as well as in North America, Europe, Asia and Oceania [1,2]. The protozoan *T. cruzi* is responsible for CD, and the primary vector of transmission occurs by the bite of the insect vector, popularly known as the kissing bug [3]. Currently, two drugs are available: benznidazole and nifurtimox. These drugs demonstrate high efficacy in the acute phase of the disease, but low activity in the chronic phase. In this study, 13 novel pyrazole-imidazoline derivatives inspired by a previously identified cysteine protease inhibitor were evaluated for their antiparasitic activity. These compounds exhibit low cytotoxicity (CC_{50} > 100 μM) and efficacy against intracellular amastigotes. Among the 13 derivatives, compound **1k** showed high activity (IC_{50} = 3.3 ± 0.2 μM), selectivity (SI= 73.9) and potency (pCI_{50}= 5.4). The efficacy of this derivative was then assessed in *T. cruzi*-infected Vero cells (see Figure 1). This derivative was tested in a 3D cardiac microtissue model, which demonstrated a substantial reduction in parasite load. Remarkably, the efficacy of benznidazole was replicated, with comparable outcomes achieved at lower concentrations. The efficacy of benznidazole and the **1k** in preventing parasite recrudescence was demonstrated, with the combination of both exhibiting an additive interaction, indicating a potential benefit for in vivo combination therapy.

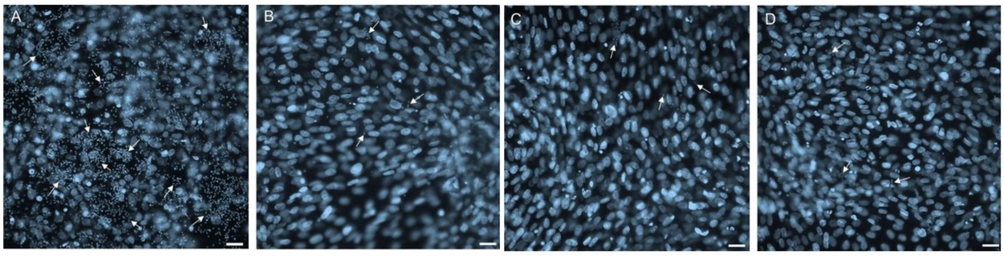

Figure 1. The effect of **1k** during a test of *washout* in infected Vero cells. **A.** untreated cultures of Vero cells for 4 days. All treated groups were infected for 24 h, treated for 10 days and then monolayers were fixed at 21 days post-infection. **B** and **C.** treatment with **1k** (28,8 μM and 57,6 μM, respectively) and **D.** treatment with benznidazole (100 μM). The arrows indicate intracellular parasites.

References

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2. Sousa, A. S.; Vermeij, D.; Ramos Jr, A. N.; Luquetti, A. O. Chagas disease. *Lancet* **2023**, 403, 203-2018. https://doi.org/10.1016/ S0140-6736(23)01787-7
3. Cucunubá, Z. M.; Gutiérrez-Romero, S. A.; Ramírez, J.; Velásquez-Ortiz, N.; Ceccarelli, S.; Parra-Henao, G.; Henao-Martínez, A. F.; Rabinovich, J.; Basáñez, M.; Nouvellet, P.; Abad-Franch, F. The epidemiology of Chagas disease in the Americas. *The Lancet Regional Health- Americas* **2024**, 37, 1-20. https://doi.org/10. 1016/j.lana.2024. 100881

Synthesis and Investigation of Fluorinated Scaffolds Exhibiting Through-Space Scalar ^{19}F –^{19}F NMR Coupling

Damiani Sara[1], Oštrek Andraž [1], Lozinšek Matic [2], Zupančič Špela [1], Peršolja Peter [1], Satta Giuseppe [3], Plavec Janez [3] and Cotman Andrej Emanuel [1]

1. University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia;
2. Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia;
3. Kemijski inštitut, Hajdrihova 19,1000 Ljubljana, Slovenia

sara.damiani@ffa.uni-lj.si

Keywords: *^{19}F–^{19}F NMR through space scalar coupling, hydrogen-bond gated systems, fluorinated molecular probes*

Fluorinated functional groups play a central role in modern medicinal and chemical research due to their strong influence on molecular conformation, physicochemical properties, and intermolecular interactions [1]. Among them, the trifluoromethyl (CF$_3$) group is particularly important [2]. In this work we report design, synthesis and characterization of α-phthalimido-trifluoromethyl carbinols as precursors of homochiral J_{FF}-detected H-bond sensors. Following a modified literature procedure on phenylalaninol [3], preliminary studies on the corresponding α-phthalimido-aldehydes revealed a selective mono-trifluoromethylation only at -78 °C, while double addition with Rupert–Prakash reagent occurs only at higher temperature. The obtained hit compound was prepared on a gram scale with a diastereomeric ratio of 44:39:7:10. One of the diastereomers displayed a peculiar pattern in the ^{19}F NMR spectrum, characterized by a pentet and a quartet corresponding to the tertiary and quaternary carbon-bound CF$_3$ group. Single-crystal X-Ray diffraction analysis confirmed close proximity of the two CF$_3$ groups and the relative (R^*,R^*,S^*) configuration revealing a hydrogen-bond-gated ^{19}F–^{19}F through-space scalar coupling [4]. Understanding the structural origin of this phenomenon could enable the rational design of fluorinated molecular systems acting as NMR-responsive probes. Therefore, motived by the use of CF$_3$ as promising probe for ^{19}F NMR [4], we started to explore β-carboxamido trifluoromethyl carbinols as potential systems in which hydrogen-bond modulation controls the presence or absence of through-space ^{19}F –^{19}F scalar coupling. Namely, solvent-dependent ^{19}F NMR experiments were performed, revealing a disruption of this hydrogen bond in DMSO-d_6/buffer mixtures and the consequent disappearance of the scalar coupling pattern, confirming the dynamic and solvent-sensitive nature of the system. Moreover, to explore potential pharmaceutical application, the hit compound was incorporated into lipid-based delivery systems to evaluate whether the characteristic ^{19}F –^{19}F coupling could still be observed in a biomimetic lipophilic environment through ^{19}F 2D NMR experiments. The results are expected to contribute to the broader understanding of fluorine-based molecular reporters and to support the development of responsive fluorinated systems for chemical and biochemical applications.

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New *O*-Prenylated Caffeic Acid Amides with Selective Toxicity Against *Trypanosoma cruzi* Amastigotes. Design, Synthesis and Structure-Activity Relationships

<u>João Sousa Janela</u>[1], Lucas de Oliveira Pires[2], Afonso Santine M. M. Velez[2], Fábio da Costa Rocha[2], Débora Decoté-Ricardo[3], Marco Edilson Freire de Lima[2], Rosane Nora Castro[2], Fernanda M. F. Roleira[1], Elisiário J. Tavares da Silva[1]

1. CERES, Universidade de Coimbra, Faculdade de Farmácia, Laboratório de Química Farmacêutica, Azinhaga de Santa Comba, Pólo III - Pólo das Ciências da Saúde, 3000-548 Coimbra, Portugal;
2. Universidade Federal Rural do Rio de Janeiro, Instituto de Química, Departamento de Química Orgânica, BR 465, Km 07, CEP: 23.890-000, Seropédica, RJ, Brasil;
3. Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Departamento de Microbiologia e Imunologia Veterinária, BR 465, Km 07, CEP: 23.890-000, Seropédica, RJ, Brasil;

joaojanela@ff.uc.pt

Keywords: *Caffeic acid amides*, *Chagas' disease*, *O-Prenylation*, *Synthesis*, *Trypanosoma cruzi*.

Chagas' disease arises as an emergent parasitosis caused by the infection with *Trypanosoma cruzi*, with only two drugs available for its treatment [1]. Cinnamic acid derivatives, such as esters and amides, are naturally occurring compounds from plant's metabolism. Their phenylpropanoid scaffold is privileged due to its structural simplicity and broad-spectrum biological properties, such as antiproliferative, antimicrobial, anti-inflammatory, antiparasitic, and antioxidant. In this context, they can be considered promising compounds in search for new drugs for Chagas' disease. Among them, caffeic acid and their esters and amides are often highlighted as the most attractive due to its assorted biological profile [2]. *O*-prenylation is described as a structural modification that benefits the crossing of cell membranes [3], which seems appropriate when designing compounds to target single-celled protozoa. Based on this, it was designed and synthesized caffeic acid amides and their respective new 4-*O*-prenylated analogues and tested their activity on amastigotes of *T. cruzi*, and their selectivity using host cells. *N*-alkylamides were more active against amastigotes in comparison to aromatic amides and displayed no toxicity against host cells. The most active compound (4-*O*-prenyl-*N*-pentylcaffeic acid amide) presented an IC_{50} of 7.54 ± 1.71 µM highlighting the importance of *O*-prenylation on the catechol moiety for both toxicity and selectivity against *T. cruzi*.

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Identification, Design, and Optimization of New Allosteric Inhibitors Against ZIKV and WNV NS2B-NS3^{pro}

Demuru Laura[1], Lupia Antonio[1], Nieddu Salvatore[1], Esposito Francesca[1], Bryant Sharon. D[2], Onali Alessia[1], Sanna Erica[1], Atzeni Giulia[1], Cottiglia Filippo[1], Emmolo Roberta[1], Corona Angela[1], Tramontano Enzo[1], Maccioni Elias[1], Meleddu Rita[1], Distinto Simona[1]

1. Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria, Monserrato, Italy;
2. InteLigand GmbH, Mariahilfer Straße 74B/11, 1070 Vienna, Austria;

laura.demuru2@unica.it

Keywords: flavivirus, ZIKV, WNV, allosteric inhibitors

Zika virus (ZIKV) and West Nile virus (WNV) are mosquito-borne members of the Flaviviridae family, recognized as emerging human pathogens. Aedes mosquitoes primarily transmit ZIKV, whereas Culex species predominantly transmit WNV. The global distribution and public health impact of these viruses underscore the urgent need for effective preventive and therapeutic strategies. The viral NS2B-NS3 protease (NS2B-NS3^{pro}) is essential for viral replication, as it cleaves the viral polyprotein into structural and non-structural components [1]. NS3^{pro} is a chymotrypsin-like enzyme featuring a catalytic triad composed of S135, H51, and D75 in its N-terminal region. The structural similarity between the NS3^{pro} active site and various host serine proteases, combined with the limited effectiveness of covalent peptide-based inhibitors, has made it difficult to develop selective competitive inhibitors, making allosteric inhibitors the preferred strategy [2]. Currently, no approved antivirals exist for ZIKV and WNV. In this study, an integrated approach combining several in silico methods with biochemical assays was employed to identify four potential inhibitors from commercial and FDA-approved compound databases targeting NS3^{pro}. Considering the high conservation among the flaviviral proteases, the compounds were also tested against ZIKV^{pro} and WNV^{pro}. Three compounds exhibited IC_{50} values below 30 μM against ZIKV^{pro}, while four showed IC_{50} values ranging from 11 to 33 μM. Based on these results, the scaffold of the active compound 47P was selected for further optimization, leading to the synthesis of a library of 42 analogues. Among the fourteen compounds tested so far, three demonstrated improved inhibitory activity against ZIKV NS3^{pro}, and four showed enhanced activity against WNV NS3^{pro}. Additionally, the known allosteric inhibitor NSC135618 was synthesized, and enzyme kinetic studies are ongoing to elucidate the mechanism of inhibition of the newly identified compounds.

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Synthesis, characterization and evaluation of anti-*T.* cruzi activity of 1-arylpyrazole-4-carboximade-*N*-propylimidazoles

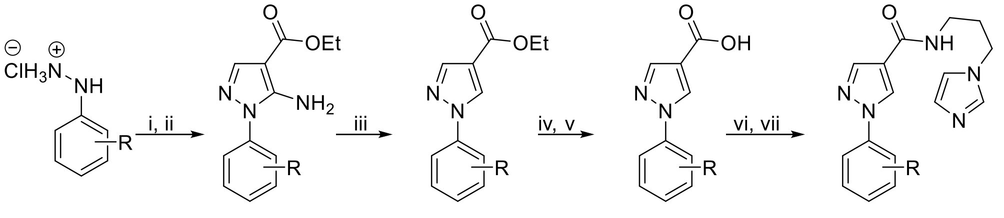
Lemos Leonardo C[1,2], Santos Maurício S. [1], Matos Maria J. [2], Paes Vitória B. [3] and Pereira Mirian C. S.[3]

1. Department of Physic and Chemistry, Federal University of Itajubá, Itajubá, 37500-903, Brazil.
2. Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain.
3. Laboratório de Ultraestrutura Celular, Instituto Oswaldo Cruz, Rio de Janeiro, 21040-900, Brazil.

leonardo.censi@rai.usc.es

Keywords: *Chagas Disease, pyrazole, imidazole*

Chagas Disease (CD), an illness first identified by Carlos Chagas 117 years ago, continues to represent a significant public health challenge. An annual total of thirty thousand new cases has been reported, resulting in fourteen thousand deaths during the same period. At the moment, only two drugs are used in the chemotherapy: benznidazole and nifurtimox. Nifurtimox is the only drug commercially available in several countries. The primary concerns regarding this drug are due to its high rate of attrition, which is estimated to be approximately 20%, largely attributable to the occurrence of substantial adverse effects. Additionally, the prolonged duration of its administration is another important issue, as is its apparent inefficacy in the chronic phase of CD [1]. In the search for new candidates with potential trypanocidal effect, our research group has been synthesizing several heterocyclic systems for their antiparasitic evaluation [2]. The main goal of this study is to synthesize and evaluate the trypanocidal activity of 13 novel 1-aryl-*N*-(3-(1*H*-imidazol-1-yl)propyl)-1*H*-pyrazole-4-carboxamides. The structures of all the compounds have been confirmed by Fourier-transform infrared spectroscopy, high-resolution mass spectrometry, and nuclear magnetic resonance analysis. Biological evaluation was conducted on the clone Dm28c in both its intracellular amastigote and trypomastigote forms. This work will present the initial chemical and biological results.



1(a-m)

R= **a:** H; **b:** 3-Cl; **c:** 3-Br; **d:** 3-F; **e:** 3-NO_2; **f:** 4-Cl; **g:** 4-Br; **h:** 4-OCH_3; **i:** 4-F; **j:** 4-NO_2; **k:** 3,5-diCl; **l:** 3,4-diCl**; m:** 3-Cl-4-CH_3

Scheme 1. Synthetic route to obtain **1(a-m)**. Reagents and conditions: i) AcONa, ethanol, 15 min; ii) ethyl-2-cyano-3-ethoxyacrylate, 1 h, reflux; iii) *t*-butyl nitrite, THF, 2 h, reflux; iv) NaOH 3,5 mol*L^{-1}, ethanol, 10 min, reflux; v) HCl 10% m/v; vi) $SOCl_2$, toluene, 70 ◦C, 2 h; vii) 3-amine-1-(1*H*-imidazol-1-yl)propane, pyridine, 70 ◦C, 2 h.

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Expanding the Therapeutic Potential of Purine C-Nucleosides from Chemical Synthesis to Oligonucleotide Incorporation

Faloci Federico [1], Ghezzo Michele [1], Groaz Elisabetta [2,3], and Sissi Claudia [1]

1. Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy;
2. Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, 3000 Leuven, Belgium;
3. Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, 3000 Leuven, Belgium

federico.faloci@phd.unipd.it

Keywords: *C-nucleoside, 4-aza-7,9-dideazapurine, phosphoramidite and oligonucleotide*

Nucleoside and nucleotide analogues represent a diverse and versatile class of compounds in medicinal chemistry, that find applications for a range of therapeutic strategies as antiviral and anticancer treatments. Within this context, C-nucleosides are particularly attractive scaffolds, where the unstable C-N glycosidic bond is replaced by a more stable C–C bond. This feature imparts enhanced chemical and metabolic stability, as demonstrated by remdesivir, an FDA-approved broad-spectrum antiviral agent [1,2]. Additionally, this structural modification can alter base-pairing geometry, providing opportunities to modulate the physicochemical properties of nucleic acids [2,3]. Despite these advantages, the development of purine-based C-nucleosides and their incorporation into oligonucleotide architecture remains relatively underexplored.

In this study, we explored the synthesis of 4-aza-7,9-dideazapurine C-nucleoside analogues, characterized by targeted modifications of the purine scaffold through N-C replacements within the heterocycle core, designed to modulate electronic distribution and intermolecular interactions. A convergent C-glycosylation approach, providing access to both adenine- and guanine-modified derivatives. In addition, selected adenosine derivatives were converted to the corresponding phosphoramidites and incorporated site-selectively into RNA sequences via automated solid-phase synthesis. This approach will provide novel purine C-nucleoside entities to be evaluated as both anticancer and antiviral therapeutic agents. Additionally, they will be used as functional building blocks to be inserted within oligonucleotide strands to assess their potential impact on the optimization of anticancer agents.

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Evaluation of mitochondrial toxicity induced by a novel COMT inhibitor

Bárbara Albuquerque[1], Carla Lima[2], Sofia Benfeito[1], Daniel Chavarria[1], Carlos Fernandes[1], Paula Serrão[1], Fernanda Borges[1,2], Patrício Soares-da-Silva[1]

1. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
2. RISE-Health, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

up201804981@up.pt

Keywords: *Brain-selective COMT inhibitor, Mitochondrial toxicity, Oxidative stress, Mitochondrial membrane potential,* and *Neurological disorders*

Catechol-*O*-methyltransferase (COMT) inhibitors are commonly used for treating Parkinson's disease (PD) and potentially useful in other neuropsychiatric pathologies characterized by low dopamine levels in the brain. Currently available COMT inhibitors face limitations such as adverse reactions and low bioavailability [1], such as tolcapone (here used as reference compound), a COMT inhibitor that acts in the central nervous system, but with reported hepatocellular injury and acute liver failure. Compound 9 is a novel, brain-selective, non-nitrocatechol COMT inhibitor developed to overcome these issues [2]. In this study, the toxicological profile of compound 9 was evaluated by assessing its effect on cell mass, oxidative stress, ATP production, and mitochondrial membrane potential (Δψm). Human neuroblastoma (SK-N-SH) and hepatocarcinoma (HepG2) cells were treated with compound 9 or tolcapone (0.3–30 µM) for 24 or 48h. Tolcapone decreased cellular viability in both cell lines, characterized by a marked reduction in cell mass and severe ATP depletion, particularly at concentrations above 10 µM. These effects were accompanied by increased oxidative stress and a biphasic Δψm response-initial depolarization followed by hyperpolarization-consistent with mitochondrial uncoupling and hepatotoxicity [3]. In contrast, compound 9 exhibited a higher safety profile. Both cell mass and ATP homeostasis were not significantly affected across the tested concentration range, with oxidative responses suggestive of cellular adaptation rather than cytotoxic damage. Furthermore, Δψm remained largely stable, indicating minimal interference with mitochondrial function. To conclude, the novel brain-selective COMT inhibitor displayed a safer mitochondrial profile than tolcapone, supporting its potential as a promising candidate for further development.

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Dual-Target Drug Discovery in Alzheimer's Disease Using Machine Learning guided approach integrated with structure based virtual screening

Asim Ayesha[1,2], Jastrzębski Michał K.[1,2], Karcz Tadeusz[2], Olejarz-Maciej Agnieszka[2], Koval Maryna[1], Kukuła-Koch Wirginia[3], A.Kaczor Agnieszka[4]

1. Doctoral School of the Medical University of Lublin, Poland;
2. Faculty of Pharmacy, Jagiellonian University, Medical College, Kraków, Poland;
3. Department of Pharmacognosy with Medical Plants Garden, Medical University of Lublin, Poland;
4. Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Laboratory, Medical University of Lublin, Poland

ayesha.asim4@gmail.com

Keywords: *Dual Inhibitors, AChE, MAO-B, Machine Learning Modeling, HTVS*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with complex and multifactorial pathology, for which current therapies provide only symptomatic relief. Dual inhibition of two key targets, acetylcholinesterase (AChE) and monoamine oxidase-B (MAO-B), has emerged as a promising strategy to concurrently enhance cholinergic neurotransmission and reduce the oxidative stress in AD [1]. In the present study, a machine learning–guided approach integrated with structure-based virtual screening was employed to identify novel small-molecule candidates as potential dual AChE and MAO-B inhibitors. Bioactivity data for both targets was retrieved from the ChEMBL database and, after curation and preprocessing, used to develop predictive machine learning models employing artificial neural networks (ANNs) and decision trees (DTs). These models were applied to virtually screen the Enamine database of approximately 0.46 million compounds, yielding 3,890 prioritized candidates. This subset was subsequently subjected to high-throughput virtual screening against X-ray structures of AChE and MAO-B. As a result, compounds showing favorable binding affinities and key interactions with critical active-site residues of both enzymes were shortlisted, yielding 11 potential dual-target candidates, alongside 18 compounds prioritized as selective MAO-B inhibitors. All the compounds were further evaluated using PAINS filtering and pharmacokinetic profiling. Experimental validation showed that compound 5 demonstrated balanced dual inhibition of AChE and MAO-B, supporting its potential as a promising lead scaffold. Additionally, selective inhibition was observed, with compound **2** exhibiting 32% AChE inhibition (berberine: 57%), and compound **10** showing 96% MAO-B inhibition comparable to safinamide (100%). Moreover, two compounds from selective MAO-B inhibitors series exhibited strong MAO-B inhibition (96–98%), approaching the activity of clinically used inhibitors such as rasagiline and safinamide. Collectively, these results validate the effectiveness of the integrated machine learning and structure-based virtual screening workflow and identify promising chemical scaffolds for the rational development of dual AChE/MAO-B inhibitors. In vivo studies, including zebrafish assays and behavioral testing in mice are currently underway to further validate the therapeutic potential of these candidates.

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Nanocarrier-Based Co-Delivery of Edaravone and Riluzole for Improved ALS Treatment Strategies

Cláudia S. Machado[1], Ana Rita Alfenim[1], Joana Moreira[1], Brandon Aguiar[2], Miguel Pinto[1], Fernanda Borges[2] and Carlos Fernandes[1]

1. RISE-Health, Faculty of Medicine, University of Porto, Al1ameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal;
2. RISE-Health, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal.

up201805412@fc.up.pt

Keywords: *ALS, edaravone, riluzole, dual-drug encapsulation, lipid-polymer hybrid nanoparticles*

Amyotrophic lateral sclerosis (ALS) is known to be fatal and the most common degenerative disease of the motoneuron system, possibly involving various genetic and molecular pathways, that still lack effective treatment. Edaravone (Edv), a free radical scavenger, and Riluzole (Ril), a glutamate release inhibitor, are two approved drugs for ALS therapy. However, these are limited as they can only target one mechanism and present biocompatibility issues [1]. Nanotechnology-based drug delivery systems arise as a powerful tool for enhancing therapeutic bioavailability by inferring drug stability and targeting the desired site [2]. Furthermore, the combination of drugs that aim different pathways in the same nanocarrier brings an advantage over the conventional methodologies as a synergy of different drugs can be achieved.

In this work, we encapsulated both Edv and Ril in different drug delivery systems using polymeric, lipidic or a mixture of both lipid and polymer core. For that, polylactic-co-glycolic acid (PLGA) nanoparticles, nanostructured lipid carriers (NLCs) and lipid-polymer hybrid nanoparticles (LPHNPs) were developed and functionalized with PEG or Vitamin E (TPGS) in order to verify the effect on the stability and interaction with cellular models. Drug delivery systems were analyzed by Dynamic and Electrophoretic Light Scattering to verify their storage stability. The interaction with mucus protein as well as the behavior of the nanocarriers in different pH media was also evaluated. The pharmacological profile of the most promising nanoformulation was evaluated in an *in vitro* cellular study using mouse motor neuron-Like (NSC-34) hybrid cell line.

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Identification of Natural Tyrosinase Inhibitors as potential therapy for Neurodegenerative Diseases

Fossati Alice[1,2], Cavalloro Valeria[1,2], Emanuela Martino[1,2], Battisti Verena[3], Langer Thierry[3], and Collina Simona[4].

1. Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy
2. National Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy
3. Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14 (UZA II), 1090 Vienna, Austria
4. Department of Drug Science, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy.

alice.fossati01@universitadipavia.it

Keywords: *Computational chemistry, Natural products, Plant extracts, Biodiversity,* and *neurodegenerative diseases.*

Neurodegenerative diseases (ND) cause progressive neuron loss with severe cognitive/motor deficits. In 2019 they affected 50M people (WHO), rising to 3.4B (43% global population) by 2021. These disorders were responsible for about 11.1 million deaths, making them the leading cause of global disease burden [1]. Tyrosinase (Tyr), known for skin hyperpigmentation, plays a critical role in neurodegeneration: it oxidizes dopamine in dopaminergic neurons, generating reactive intermediates, cytotoxic neuromelanin, and oxidative stress, causative of α-synuclein misfolding, mitochondrial dysfunction, and neuronal apoptosis. Moreover, Tyr overexpression in aging/stress amplifies pathological cascades [2]. Tyr inhibitors are approved only for cosmetic purposes and currently their application as neurodegeneration therapies is currently underrated. In this context, plants produce secondary metabolites with multi-target neuroprotective effects, long used in traditional medicine and now promising for ND prevention. Moreover, no systematic studies exist on human-specific Tyr inhibitors from local flora. This study aimed to identify natural products with potential therapeutic benefits for ND. In particular, twenty-seven plants belonging to different botanical families were selected using a taxonomic approach within the NBFC project framework and screened in vitro for tyrosinase inhibition [3]. *Carissa macrocarpa* (Eckl.) A.DC. and *Adenophora lilifolia* (L.) Ledeb. ex A.DC. extracts demonstrated the most promising inhibitory activity. A comprehensive database of secondary metabolites from these plants was built based on literature and UHPLC-MS phytochemical characterization data. A pharmacophoric model for Tyr inhibitors was developed and validated to screen this database. The resulting hits were subjected to molecular docking analysis towards binding pockets of both *Agaricus bisporus* (J.E. Lange) Imbach tyrosinase and a human homology model of tyrosinase. Our findings establish a starting point for identifying specific natural products that effectively inhibit Tyr, enabling the preparation of plant extract and isolation of pure compounds with potentially enhanced efficacy against ND diseases.

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Chasing NOX4: From VAS2870 to a Potent, Selective, Covalent Inhibitor

Mariana Castelôa [1], Marta Massari [2], Sara Marchese [2], Carla Lima [1], Joana Reis [2,3], Lorenzo Basile [2], Fernanda Borges [1,4], Sofia Benfeito [4], Andrea Mattevi [2], Daniel Chavarria [4]

1. RISE-Health, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
2. Department of Biology and Biotechnology Lazzaro Spallanzani, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy
3. Department of Structural Biology, St. Jude Children's Research Hospital, 262 Danny Thomas Pl, 38105 Memphis, TN, USA
4. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

up201606405@fc.up.pt

Keywords: *NADPH oxidases, homolog selectivity, targeted covalent inhibitors,* and *cysteine-targeting.*

The NADPH oxidase (NOX) family comprises seven homologs (NOX1-5; DUOX1-2) that generate reactive oxygen species (ROS) and represent promising therapeutic targets in oxidative stress-related diseases. However, developing selective NOX inhibitors remains a major challenge due to the high structural conservation of the enzymes' catalytic cores. Among known NOX inhibitors, VAS2870 stands out as a *bona fide*, non-assay-interfering compound that acts through covalent binding to a cysteine residue in the dehydrogenase domain. However, it shares common limitations with other NOX inhibitors, including lack of homolog selectivity, poor aqueous solubility, and cytotoxic effects [1,2]. In this work, a library of VAS2870 derivatives bearing distinct cysteine-reactive warheads was designed, synthesized, and screened against full-length NOX1, NOX2, NOX4, and NOX5. From this effort, a promising hit was identified. Subsequent hit-to-lead optimization, consisting of linker modification while maintaining the electrophilic warhead, was performed to improve potency and selectivity while confirming the utility of the chosen warhead. Ultimately, this optimization led to the discovery of a potent, non-interfering, selective NOX4 inhibitor with a favorable cytotoxicity profile. The results obtained will be presented in this communication.

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Pharmacomodulation of 2-Amidobenzothiazoles for the Development of Neuroprotective Compounds: A Phenotypic Study in an Alzheimer's Disease Model

Novás Manuel[1,2], Martínez Antón L. [2], Loza María Isabel [2], and Matos Maria J. [2]

1. BioFarma Research Group, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain;
2. Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain;

manuel.novas@usc.es

Keywords: *neuroprotection, Alzheimer's, benzothiazole and SH-SY5Y*

The benzothiazole scaffold and its analogs are present in several clinically approved central nervous system drugs with neuroprotective properties, including riluzole, pramipexole (and dexpramipexole), and lurasidone. Inspired by the therapeutic potential of this privileged structure, we designed and synthesized a library of 48 novel 2-amidobenzothiazole derivatives through systematic pharmacomodulation of the scaffold. Structural diversity was introduced by incorporating a broad range of electron-donating and electron-withdrawing groups, as well as substituents of varying steric demand, in order to expand the explored chemical space. Compounds were then screened in a phenotypic Alzheimer's disease model of SH-SY5Y cells transfected with the mutations *MAPT* P301L and *APP* V717I, which induce the formation of pathological protein aggregates [1]. A high-throughput screening calcium influx assay and neurite length immunostaining were performed to determine the neuroprotective effect of the compounds. Screening of the compound library identified derivatives capable of producing a dose-dependent protective effect, reversing neurite shortening and attenuating calcium influx alterations in the cellular model. Physicochemical profiling revealed that trifluoromethyl and trifluoromethoxy substituents significantly modulate compound solubility, which in turn influences the observed biological activity [2]. The study of this family of 48 new compounds has helped establish interesting structure-activity relationships, which may enable further optimization of the scaffold in the hit-to-lead process.

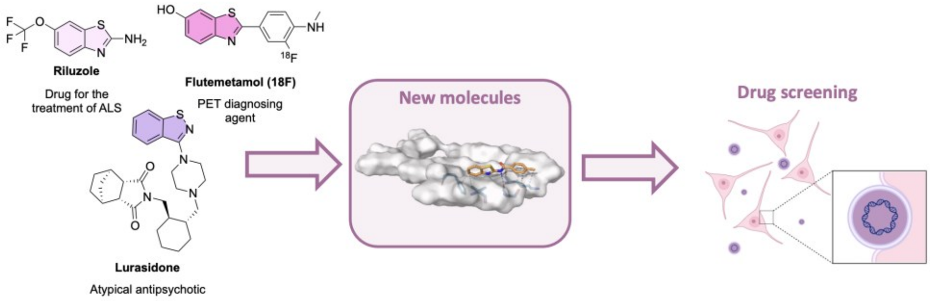

Figure 1. Diagram of the workflow of the project.

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Targeting Mitochondrial Iron Dysregulation in Friedreich’s Ataxia Using Novel Chelators

Carla Lima[1], Mariana Casteloa[1], Daniel Chavarria[2], Lisa Sequeira[2], Fernando Cagide[2], Fernanda Borges[1,2], Sofia Benfeito[2]

1. RISE-Health, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
2. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

carlapclima@gmail.com

Keywords: *Ferroptosis, Friedreich’s Ataxia, Iron chelation, Mitochondrial dysfunction*

Friedreich's Ataxia (FRDA; ORPHA:95) is an inherited and progressive neurodegenerative disease, being characterized by sensory loss and hypertrophic cardiomyopathy. It represents the most common form of inherited ataxia with an autosomal-recessive inheritance pattern, affecting 1 in 50.000 individuals worldwide. The disease is typically diagnosed in childhood (2-3 years) or early adulthood (>25 years) and often progresses into a fatal outcome. FRDA is associated with reduced levels of 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. Deferiprone (DFP), an iron chelator, has demonstrated potential in managing FRDA by crossing physiological barriers and targeting intracellular iron pools. However, DFP lacks mitochondrial specificity and results from its phase II trial for FRDA (NCT00897221) remain unpublished. So, we hypothesize that selectively targeting iron chelators to mitochondria can prevent iron-induced toxicity, mitochondrial dysfunction and ferroptosis. In this way, a new library of compounds based on DFP scaffold was synthesized to improve mitochondrial targeting, combining iron-chelating and antioxidant properties to address mitochondrial dysfunction and prevent ferroptosis in FRDA. After structural characterization, the compounds were evaluated for cytotoxicity in various cell lines, followed by assessment of their ability to reverse damage induced by ferroptosis. The results obtained so far will be presented in this communication.

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Beyond the 1:1 Ligand-Protein Paradigm: An In Silico Assay for Competitive Ligand Binding

Cardenas Vince Bart [1], Stefano Raniolo [1], Paolo Conflitti [1], and Vittorio Limongelli [1]

1. Euler Institute, Faculty of Biomedical Sciences, Università della Svizzera italiana, Lugano CH-6900, Switzerland

vince.bart.cardenas@usi.ch

Keywords: *Ligand-Protein Binding, Molecular Dynamics, Martini Coarse-Grained*

Competitive binding assays (CBAs) are central to pharmacology and drug discovery, providing metrics of ligand affinity and potency [1]. Yet they only provide limited mechanistic insight into the molecular events underlying ligand competition. How multiple ligands dynamically explore receptor surfaces, compete for access, and influence one another's dynamics remain experimentally difficult to resolve. All-atom molecular dynamics (AAMD) simulations potentially bridge this gap, but atomistic simulations of true competitive binding environments remain computationally prohibitive as they require multiple freely diffusing ligands, long timescales, and extensive sampling [2]. Here, we present a computational framework of CBA-like conditions using fully unbiased, multiligand coarse-grained molecular dynamics (CGMD) simulations [3], applied to ligands of opposing efficacy, the full agonist NECA and inverse agonist ZM241385, at the adenosine A2A receptor, a prototypical GPCR and key pharmacological target [4]. By simulating varying ligand ratios, a concentration-dependent competition is emulated, while allowing both ligands to diffuse freely, explore the receptor and membrane, and interact dynamically amongst themselves. The protocol captures hallmark features of CBA, including spontaneous binding, unbinding, and direct competition across an aggregate millisecond simulation timescale, enabling structural, mechanistic, thermodynamic, and kinetic characterization inaccessible to conventional experiments. Without imposing prior binding poses, both ligands found their experimental binding modes and reproduced their experimental relative binding energies. Moreover, the simulations revealed an extracellular vestibular region that hosts transient ligand-receptor binding, accommodate ligand-ligand interactions, and modulate ligand access to the orthosteric site. Occupancy by NECA in this region facilitated ZM241385 pocket entry and prolonged its residence time, revealing a cooperative mechanism in an otherwise competitive process.

These findings provide a molecular perspective on ligand competition and highlight CGMD's promising potential for probing multiligand dynamics beyond the reach of conventional assays. More broadly, it opens new avenues towards understanding how competitive-cooperative processes can shape binding pathways, kinetics, and outcomes in drug discovery.

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Investigation of novel guaiacol-bearing molecules as multi-targeted directed ligands (MTDLs) against Alzheimer's disease-related targets

Deruvo Caterina[1], Rosa Purgatorio[1], Marianna Ranieri[2], Modesto de Candia[1], Marco Catto[1], Orazio Nicolotti[1], Grazia Tamma[2], Cosimo D. Altomare[1]

1. Department of Pharmacy-Pharmaceutical Sciences,
2. Department of Biosciences Biotechnologies and Environment, University of Bari Aldo Moro, Via E. Orabona 4, 70125, Bari,Italy

caterina.deruvo@uniba.it

Keywords: *guaiacol, multitarget-directed ligands, neuroprotection, oxidative stress, structure–activity relationships*

Natural phenolic compounds such as eugenol, vanillin, curcumin and ferulic acid (FA) share a guaiacol fragment associated with antioxidant, anti-inflammatory, and neuroprotective activities, which make it a promising moiety of multitarget-directed ligands (MTDLs) for treating neurodegenerative disorders, including Alzheimer's disease (AD) [1]. In this study, the guaiacyl moiety was conjugated with indole and isatine cores, as well as tetrahydroazepino-indoles and chromenopyridines previously disclosed by some of us as inhibitors of β-amyloid aggregation, cholinesterases and monoamine oxidases [2,3]. Herein, the activities toward the AD-related targets of the newly synthesized guaiacyl derivatives **2-5** were investigated and compared with the ferulic acid ethyl ester **1** (Figure 1).

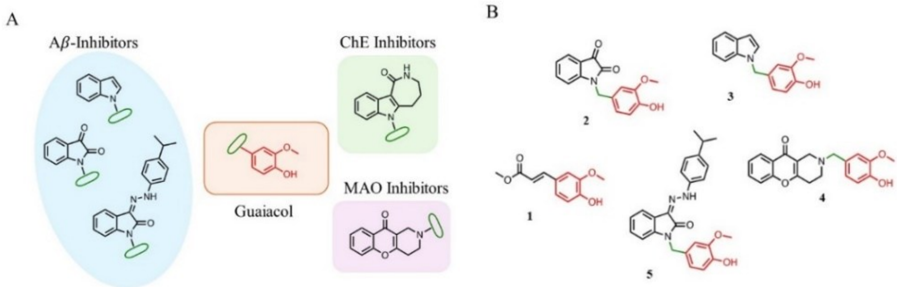

Figure 1. A. Guaiacyl derivatives targeting neurodegenerative pathways. B. New guaiacol–conjugated derivatives.

The biological evaluation includes inhibition assays of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidases A and B (MAO A and B), β-amyloid aggregation studies, radical scavenging activity, and intracellular ROS measurements in cell models under oxidative stress conditions.

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Prodrug strategies to improve the drug-like properties of fragment-derived Keap1-Nrf2 Inhibitors

Fabiana Lo Mascolo[1], Paola Barraja[1], Anders Bach[2]

1. Department of Sciences and Chemical Biology and Pharmaceutical Technology (STEBICEF), University of Palermo, Via Archirafi 32, 90123 Palermo, Italy;
2. Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark;

fabiana.lomascolo@unipa.it

Keywords: *Keap1-Nrf2 pathway, Fragment-Based Drug Discovery, Prodrug design, ROS*

Oxidative stress is a key driver of neurodegenerative, inflammatory and renal diseases [1]. Pharmacological activation of the Nrf2 pathway via disruption of the Keap1-Nrf2 protein-protein interaction is a promising therapeutic strategy; however, most non-covalent Keap1 inhibitors rely on carboxylic acid moieties required for binding to the arginine-rich pocket, often resulting in poor membrane permeability and suboptimal drug-like properties [2,3]. To overcome this limitation, we investigated a prodrug approach to transiently mask the carboxylic acid functionality of fragment-derived Keap1 inhibitors identified through fragment-based drug discovery. A focused library of 26 prodrugs was synthesized and evaluated for aqueous solubility (PBS, pH 7.4, 0.5-4% DMSO; 30-200 μM) and passive permeability using PAMPA. Quantitative LC-MS methods based on selective ion monitoring (SIM) were developed for sensitive compound detection. Initial screening identified three methyl ester derivatives with markedly improved permeability, reaching Pe (cm/s) values up to 1.16×10^{-5} cm/s versus $\leq 8.62 \times 10^{-7}$ cm/s for parent acids (~13-fold increase), including complete rescue from undetectable to high permeability. Building on these results, corresponding thiazolidinone-based prodrugs were designed and synthesized to further optimize physicochemical properties and enable ROS-responsive activation. These analogues retained favorable solubility and permeability profiles and were subjected to oxidative activation studies. LC-MS kinetic analysis confirmed concentration-dependent oxidative cleavage. Under mild oxidative conditions (1 mM H_2O_2), prodrugs showed gradual and controlled degradation, with 56-68% remaining after 180 min, whereas faster conversion occurred at higher ROS levels, indicating a tunable balance between stability and activation. Overall, this study demonstrates that prodrug strategies can effectively address permeability limitations of fragment-derived Keap1 inhibitors while enabling ROS-triggered activation. Selected compounds are currently under biological evaluation in cellular models of Nrf2 activation in collaboration with Prof. Olagnier's laboratory (Aarhus University). Supporting further development of drug-like modulators of the Keap1-Nrf2 pathway.

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Towards Closed-Loop Spatiotemporal Treatment of Drug-Resistant Epilepsy with Photopharmacology

Tack Ruben[1], Vergaelen Marijke[2], Guerrero Ancil Javier[2], Raedt Robrecht[2] and Van Calenbergh Serge[1]
1. Laboratory of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Belgium;
2. Brain, Faculty of Medicine & Health Sciences, Ghent University, Belgium;
ruben.tack@ugent.be

Keywords: *coumarin, photocaging, spatiotemporal drug release* and *drug-resistant epilepsy*

Photopharmacology holds significant promise for the treatment of neurological diseases such as epilepsy, a condition affecting approximately 50 million people worldwide, of whom nearly 30% suffer from a drug-resistant form. Activation of the adenosine A1 receptor (A1R) is well established as a mechanism for suppressing neuronal excitability and seizure activity. However, systemic administration of A1R agonists, including N^6-cyclopentyladenosine (CPA), has failed clinically due to unacceptable peripheral side effects arising from the ubiquitous expression of A1Rs throughout the body. To overcome these limitations, a photocaged derivative of CPA (cCPA) was designed using 7-diethylamino-4-hydroxymethylcoumarin (7-DEACM) as a photolabile protecting group. This strategy enables light-triggered release of the A1R agonist, allowing on-demand, spatially confined activation of A1R in seizure-relevant brain regions such as the hippocampus. The feasibility of delivering CPA in a closed-loop controlled manner has been demonstrated in both *ex* and *in vivo* settings, with the latter employing intracerebroventricular administration of cCPA [1]. While these results are encouraging, the 7-DEACM photoprotecting group carries notable limitations. Its photorelease requires near-UV light (405 nm), which penetrates biological tissue poorly and carries a risk of cytotoxicity. Furthermore, 7-DEACM exhibits limited aqueous solubility, constraining its applicability in physiological settings.To address these shortcomings, the uncaging wavelength of the coumarin was red-shifted towards a more biocompatible range and aqueous solubility was improved by introducing distinct hydrophilic side chains at the 7-position of the coumarin core. Additionally, incorporation of a methyl group at the 4-position was pursued to improve uncaging kinetics, thereby further mitigating the risk of phototoxicity in the brain. Figure 1 illustrates the aforementioned structural optimizations incorporated into the coumarin photocage. The resulting caged anti-epileptics were systematically characterized for their aqueous solubility and uncaging kinetics. Following confirmation of efficient photorelease, their anticonvulsant potential was evaluated in acute hippocampal brain slices prepared from an intrahippocampal kainic acid induced mouse model of temporal lobe epilepsy, using multielectrode array recordings to assess seizure suppression upon light-triggered agonist release [2]. Together, these improvements represent a meaningful step towards a clinically viable photopharmacological strategy for the on-demand, spatiotemporally controlled treatment of drug-resistant temporal lobe epilepsy.

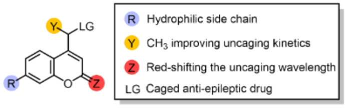

Figure 1. Optimized coumarin scaffold highlighting key structural modifications to improve photopharmacological properties.

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Targeting the vimentin-G4 repeats axis to disrupt EMT and cancer cell invasion

Dal Ponte Nicolò[1], Cozzaglio Marta[1], Rotondo Martina[2], Biondi Barbara[2], Rigo Riccardo[1], and Sissi Claudia[1]

1. Department of Pharmaceutical and Pharmacological Science, University of Padova, via Marzolo 5, 35131, Padova, Italy;
2. Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131, Padova, Italy.

nicolo.dalponte@phd.unipd.it

Keywords: *G-quadruplexes, G4 repeats, Vimentin, EMT,* and *metastasis*

G-quadruplexes (G4s) are non-canonical nucleic acid secondary structures that can arise at guanine-rich tracts, mainly enriched at telomeres and gene promoters. When multiple G4 units are located nearby, an intramolecular crosstalk can occur, leading to the formation of higher-order structures called G4 repeats (G4rps). Despite their biological relevance, only a few proteins have been identified as G4rps binding partners. We recently showed that the cytoskeletal protein vimentin selectively recognizes these arrangements over single G4s and other DNA structures [1]. Vimentin is a type III intermediate filament protein, mainly present in the cytoplasm, where it is arranged into filaments. In some cancer cells, it can also be found in the nucleus as soluble tetramers. Beyond its structural role, vimentin is a key regulator of the Epithelial-to-Mesenchymal Transition (EMT), a process that promotes cell invasion and supports metastasis in several tumor types. In these malignant cells, vimentin is generally overexpressed, highlighting its potential as a therapeutic target for preventing cancer development [2]. Although no structural data of the vimentin-G4rps complex are currently available, this system represents a novel and attractive pharmaceutical target. Here, we investigate the molecular basis of the vimentin-G4rps interaction using a “divide and conquer” approach, targeting both the protein and DNA domains involved in this interaction. By combining Hydrogen-Deuterium Exchange Mass Spectrometry and Surface Plasmon Resonance, we identified a region within the vimentin N-terminal domain that is actively responsible for G4rps binding [3]. A subsequent alanine-scanning approach revealed key residues driving this interaction, enabling the design of mutated vimentin variants with tuned affinity for G4rps. Regarding the DNA counterpart, screening a series of novel G4 binders led to the identification of a promising G4 ligand that will serve as a scaffold to develop new derivatives selective for G4rps over single G4s [4]. In parallel, a panel of vimentin binders was evaluated for their ability to modulate its polymerization state, potentially stabilizing one of its structural forms (i.e., filaments or tetramers), and to impair the vimentin-G4rps interaction. Overall, this work sheds light on previously unexplored protein-DNA interaction and paves the way to innovative strategies to interfere with EMT-related processes.

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New PROTAC approaches targeting the pseudokinase ILK

Marta Durán Martínez,[1]José Luis Aceña,[1]Laura Calleros,[2]Sergio de Frutos García, Diego Rodríguez Puyol,[3]Javier García Marín[1]

1. Departamento de Química Orgánica y Química Inorgánica, Universidad de Alcalá (IRYCIS), RICORS-2040, Instituto de Investigación Química "Andrés M. Del Río" (IQAR), INNOREN-CM, 28805-Alcalá de Henares, Madrid, Spain.
2. Departamento de Biología de Sistemas, RICORS 2040, Fundación Renal Iñigo Álvarez de Toledo, INNOREN-CM, Universidad de Alcalá (IRYCIS), (IQAR), 28805-Alcalá de Henares, Madrid, Spain.
3. Departamento de Medicina, Universidad de Alcalá, Servicio de Nefrología del Hospital Príncipe de Asturias, Instituto Ramon y Cajal de Investigación Sanitaria, RICORS 2040, Fundación Renal Iñigo Álvarez de Toledo, INNOREN-CM, Alcalá de Henares, 28871 Madrid, Spain

m.duranm@uah.es

Keywords: ILK, PROTAC, chronic kidney disease, cancer

Pseudokinases represent an underexplored class of proteins with key regulatory roles in cell signaling. Notably, no proteolysis-targeting chimeras (PROTACs) have been reported to date for pseudokinases, highlighting a significant gap in the field of targeted protein degradation. Integrin-linked kinase (ILK), a well-characterized pseudokinase, is implicated in multiple pathological processes, including cancer and chronic kidney disease, and provides an attractive model to address this challenge and explore its potential as therapeutic target.

In this work, we describe the design and synthesis of a novel PROTAC aimed at inducing the selective degradation of ILK via the ubiquitin–proteasome system. The proposed molecule combines an ILK-binding ligand with an E3 ligase recruiter through a rationally designed linker to promote proximity-driven ubiquitination and subsequent degradation of the target protein.

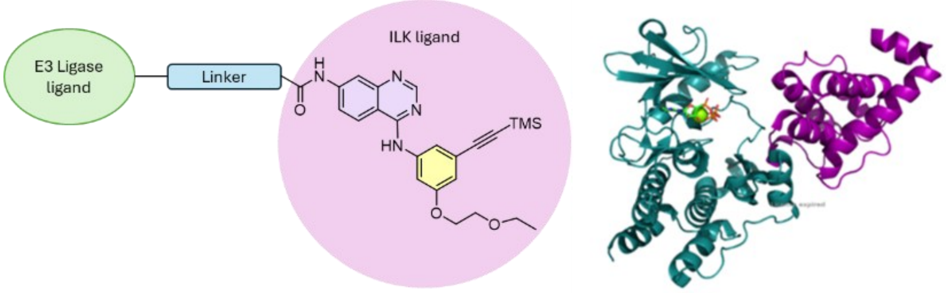

Figure 1: ILK PROTAC design

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Targeting Mitochondrial $K_V1.3$ in Cancer: Development of Mitochondria-Directed Inhibitors

Marzia Fois[1], Natalija Trunkelj[1], Jaka Dernovšek[1], Maša Omerzel[2], Boštjan Markelc[2], Joshua A. Nasburg[3], Caterina Sturtzel[4], Martin Distel[4,5], Heike Wulff [3], Luis Pardo[6], Lucija Peterlin Mašič[1], Tihomir Tomašič[1]

1. University of Ljubljana, Faculty of Pharmacy, Askerceva cesta 7, 1000 Ljubljana, Slovenia
2. Oncology Institute, Department of Experimental Oncology, Zaloska cesta 2, 1000 Ljubljana, Slovenia
3. University of California, Davis, Department of Pharmacology, 451 Health Science Drive, Davis, CA 95616, USA
4. Children's Cancer research Institute, Zimmermannplatz 10, 1090 Wien, Austria
5. Division of Pediatric Hematology and Oncology, Intermountain Primary Children's Hospital, Huntsman Cancer Institute, Spencer Fox Eccles School of Medicine at the University of Utah, 2000 Circle of Hope Dr., Salt Lake City, 84112, UT, USA
6. AG Oncophysiology, Max-Planck Institute for Experimental Medicine, Hermann-Rein- Str. 3, 37075 Gottingen, Germany

marzia.fois@ffa.uni-lj.si

Keywords: cancer, apoptosis, mitochondrial $K_V1.3$, triphenylphosphonium and pyridine

The voltage-gated potassium channel $K_V1.3$ is expressed both at the plasma membrane and in the inner mitochondrial membrane, where it plays a key role in cell proliferation and apoptosis. Mitochondrial $K_V1.3$ (mito$K_V1.3$) has emerged as a promising anticancer target, although selective compartment targeting remains challenging.[1] This work focuses on the development of mitochondria-targeted $K_V1.3$ inhibitors and the identification of a novel scaffold for plasma membrane $K_V1.3$, providing a basis for the development of new mitochondria-targeted inhibitors. Starting from a benzamide-based scaffold[2], analogue series were designed by combining linker optimization, stereochemical modulation, and incorporation of mitochondrial targeting moieties (MTMs). Structure-activity relationship studies highlighted the importance of linker length and configuration, with five-carbon linkers and *cis* isomers showing improved activity. Different targeting strategies were explored, including triphenylphosphonium (TPP^+), *para*-substituted TPP^+ analogues and pyridine-based MTMs. Selected compounds displayed potent antiproliferative activity, induced mitochondrial dysfunction and apoptosis, were active in advanced models, including 3D spheroids and organoids, zebrafish xenografts, and a mouse tumour model. In parallel, ligand-based virtual screening identified a new scaffold, which was further optimized to generate new $K_V1.3$ inhibitors and subsequently functionalized for mitochondrial targeting. Preliminary results confirmed activity, while studies on mitochondrial targeting and selectivity are currently ongoing. Overall, this work supports the combination of scaffold diversification and mitochondrial targeting as a strategy for next-generation $K_V1.3$ anticancer agents.

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Integrating Medicinal Chemistry and Bioinformatics to Advance Knowledge on P2X7R in Neuroinflammation and Tumor Immunity

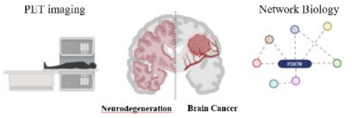
Angela Santo[1], Imane Ghafir El Idrissi[1], Daniele Vitone[1], Diego Dal Ben[2], Andrea Spinaci[2], Michela Buccioni[2], Rosaria Volpini[2], Jens D. Mikkelsen[3], Stefan Mordalski[4], Lars Juhl Jensen[4], Marcello Leopoldo[1], Enza Lacivita[1]

1. Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70125 Bari (Italy);
2. Scuola di Scienze del Farmaco, Università di Camerino, Via Madonna delle carceri, 62032 Camerino (Italy);
3. Neurobiology Research Unit, University Hospital Rigshospitalet, Copenhagen 2100, Denmark Institute of Neuroscience, University of Copenhagen, Copenhagen 2200, Denmark;
4. ZS Associates A/S, Lottenborgvej 26, 2800 Kgs. Lyngby (Denmark)

angela.santo@uniba.it

Keywords: *P2X7, Neuroinflammation, PET, Cancer, Bioinformatic.*

The purinergic receptor P2X7 (P2X7R) is an ATP-gated cation channel involved in cell death, cytokine release, and immune regulation. Its high expression in microglia and central role in neuroinflammation make it a promising target for Positron Emission Tomography (PET) imaging in neurodegenerative diseases [1]. Despite the development of selective antagonists, the lack of CNS-permeable molecules and limitations related to metabolic stability, brain penetration, and receptor polymorphisms have so far prevented the identification of a clinically validated radiotracer [2]. Starting from a literature survey, three P2X7R-active chemotypes were identified and evaluated for key attributes for CNS penetration. The most tractable scaffold was further modified to identify a potential P2X7 PET tracer. We identified derivative ANS-20, which combined high affinity and selectivity for human P2X7R, metabolic stability, low nonspecific binding to brain tissue, thus proposing as promising PET candidate. P2X7R also plays a complex role in cancer, contributing to both tumor progression and immune modulation [3]. Thus, a pan-cancer analysis integrating RNA-seq, co-expression networks, and enrichment analysis revealed that P2X7R is overexpressed in several tumors, particularly gliomas, where it participates in a coordinated purinergic module associated with proliferation, aggressiveness, and immune remodeling. The identification of co-expressed and potentially druggable genes suggests that targeting purinergic system with a multitarget strategy can be more effective than a single-target modulation.



This work integrates medicinal chemistry, molecular imaging, and systems biology to redefine the role of P2X7R as a diagnostic and therapeutic target.

Figure 1. P2X7R as a diagnostic and therapeutic target

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Chemical Probes for Mono-ADP-Ribosylating enzymes (Mono-ARTs) inhibition and degradation

Brogi Francesco[1], Juho Alaviuhkola[2], Lari Lehtiö[2], Tabarrini Oriana [1]

1. Department of Pharmaceutical Sciences, University of Perugia, Italy
2. Faculty of Biochemistry and Molecular Medicine, Biocenter Oulu, University of Oulu, Finland

francesco.brogi@dottorandi.unipg.it

Keywords: *PARPs, Selective inhibitors, Targeted Protein Degradation (TPD), PROTACs, Chemical Probes*

Human ADP-ribosyltransferases (ARTs), PARP enzymes and tankyrases, form a family of 17 proteins that catalyze ADP-ribosylation, a post-translational modification of proteins that regulates numerous cellular processes[1]. Poly-ARTs(PARP1-2) are well characterised, and six PARP1 inhibitors are in clinical use as anticancer agents, with a mechanism of action based on the synthetic lethality principle. The first of these inhibitors, Olaparib was approved in 2014, representing a success in the field of the precision medicine[2]. In contrast, mono-ARTs (PARP3–17), the discovery of which began in 2010 with PARP10, remain largely unexplored despite growing evidence of their involvement in oncological, immune, infectious and neurodegenerative diseases[3]. This project aims to develop selective PARPs inhibitors as chemical probes to elucidate their biology and validate them as innovative therapeutic targets. Starting from OUL232 (figure 1), the most potent and selective PARP10 inhibitor reported to date[3], we have designed and synthesized a large series of [1,2,4] triazolo[3,4-b]benzothiazole derivatives in an attempt to achieve selectivity especially towards PARP7, PARP11 or PARP14, which are potential immuno-oncology targets. In particular, PARP14 controls inflammatory pathways and interferon responses, PARP7 modulates interferon signaling and nuclear receptors such as estrogen and androgen receptors, while PARP11 regulates interferon signaling and promotes immunosuppressive tumor environments[1]. In parallel, we are also preparing PROteolysis TArgeting Chimeras (PROTAC) and Hydrophobic Tagging (HyT) derivatives, to investigate whether PARP degradation could provide therapeutic advantages. The presentation will cover the design and synthesis of PARP inhibitors and protein degraders, PARP inhibition, and crystallographic studies.

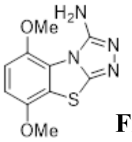

Figure 1. Structure of PARP10 inhibitor OUL232

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BRAC ligands: novel small molecules targeting the HuR-RNA complexes with a broad-spectrum anticancer activity

Garbagnoli Martina,[1,] Fontana Anna,[1] Cavassi Elena,[2] Listro Roberta,[1] Rossino Giacomo,[1] Rossi Daniela,[1] Linciano Pasquale,[1] Vaccari Melania Elettra,[1,2] Bedeschi Martina,[2] Campagnoli Lucrezia Irene Maria,[1] Marchesi Nicoletta,[1] Pascale Alessia,[1] Ambrosio Francesca Alessandra,[3] Costa Giosuè,[4] Alcaro Stefano[3,4], Gado Irene,[5] Vasile,[5] A. Tesei,[2] S. Collina[1]

1. Department of Drug Sciences, Section of Medicinal Chemistry, University of Pavia, Pavia 27100, Italy Affiliation
2. Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", 47014, Meldola, Italy
3. Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", 88100, Catanzaro, Italy
4. Associazione CRISEA - Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, 88055 località Condoleo di Belcastro (CZ), Italy
5. Department of Chemistry, University of Milan, Milano 20133, Italy

martina.garbagnoli01@universitadipavia.it

Keywords: *RNA-bonding protein, anticancer, HuR, broad-spectrum, small molecules*

RNA-binding proteins (RBPs) play a key role in regulating RNA stability, fate and function with a high impact on gene expression. Among them, the Hu proteins are the most extensively studied. Specifically, HuR is involved in proliferation, differentiation and angiogenesis and it is frequently overexpressed in various types of cancers [1]. Therefore, interfering with the formation of the HuR-RNA complex represents a promising anticancer strategy [2]. In this context, our research group identified new HuR hit compounds [3], and we have now developed a new series of HuR ligands following a fragment-based drug discovery approach. This approach used protein templated dynamic combinatorial chemistry (pt-DCC) to systematically derivatize one of our previously identified hits, characterized by a benzyl resorcinol amidic core (BRAC) The compounds have been synthesised, and their anticancer properties have been evaluated on a wide panel of cancer cells, including glioblastoma, breast and pancreatic cancer models. Depending on the modifications applied on the scaffold, we were able to identify the most promising modifications for the enhancement of the anticancer activity. Therefore, we identified a series of BRAC ligands characterized by a broad anticancer activity.

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Conjugated NOD1/PRR Agonists Reveal Distinct *In Vitro* and *In Vivo* Immune Signatures

Emiliano Paradiso[1], Špela Janež[1], Veronika Weiss[1], Ruža Frkanec[2], Žiga Jakopin[1]

1. University of Ljubljana, Faculty of Pharmacy, Askerceva cesta 7, 1000 Ljubljana, Slovenia;
2. Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, 10000 Zagreb.

Emiliano.Paradiso@ffa.uni-lj.si

Keywords: *NOD1, PRRs, PBMCs, adjuvants,* and *in vivo*

Pattern recognition receptors (PRRs), including Toll-like (TLRs), NOD-like (NLRs), and RIG-I-like receptors (RLRs), are central to detecting pathogen-associated molecular patterns and initiating innate immune responses [1]. While individual PRR activation initiates inflammatory cascades, synergistic crosstalk between distinct receptors can amplify signaling and generate unique immune signatures [2,3]. In this study, we report the design and synthesis of novel dual PRR agonists achieved by covalently linking a NOD1-selective ligand to agonists of TLR4, TLR7, or RIG-I. *In vitro* evaluations revealed that while unlinked agonist mixtures (notably NOD1 + TLR4) exhibited synergistic cytolytic activity, the covalent conjugates did not show significantly improved potency over the individual agonists. However, this activity profile shifted dramatically *in vivo*. In a murine vaccination model using ovalbumin (OVA) as the antigen, the conjugates demonstrated robust adjuvant efficacy despite their modest *in vitro* performance. Notably, the NOD1/TLR7 conjugate elicited the most pronounced systemic immune response, generating higher OVA-specific IgG titers than both the individual NOD1 ligand and the standard adjuvant, Alum. The profound *in vivo* adjuvant activity of these conjugates, contrasting with their attenuated *in vitro* potency, suggests that pharmacokinetic factors, such as enhanced cellular uptake, altered biodistribution, or the targeted, temporal release of agonists, play a critical role in their mechanism of action.

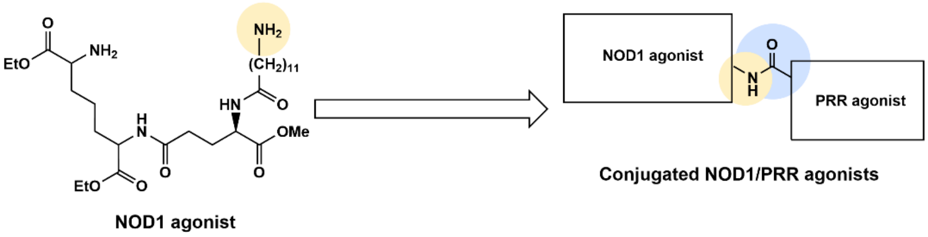

Figure 1. Design of conjugated NOD1/PRR agonists.

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Targeting m6A Epitranscriptomic Machinery: Design and Biological Characterization of Potent Quinazoline-Derived METTL3–METTL14 Inhibitors

Fabbrizi Emanuele[1], Fiorentino Francesco [2], Mancini Andrea[1], Caflisch Amedeo[3], Fatica Alessandro[2], Rotili Dante[4], and Antonello Mai[1].

1. Department of Drug Chemistry and Technologies, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy;
2. Department of Biochemical Sciences, Sapienza University of Rome, 00185 Rome, Italy;
3. Department of Biochemistry, University of Zurich, Zurich, Switzerland;
4. Department of Science, Roma Tre University, 00146 Rome, Italy.

emanuele.fabbrizi@uniroma1.it

Keywords: *Epigenetics, Epitranscriptomics, METTL3/14, RNA Methylation.*

Epitranscriptomics involves dynamic, reversible RNA modifications regulating splicing, nuclear export, stability, and translation without altering sequence. N6-methyladenosine (m6A) is the most abundant internal modification in eukaryotic mRNA and is installed by a multicomponent methyltransferase complex whose catalytic core is the METTL3/METTL14 heterodimer [1]. METTL3 contains the SAM-dependent catalytic domain with the conserved DPPW motif, whereas METTL14 acts as an RNA-binding scaffold enabling substrate recognition and positioning [2]. Dysregulation of this complex promotes tumorigenesis by enhancing stability and translation of oncogenic transcripts such as c-MYC, supporting malignant growth, thus representing a therapeutic target [3]. We report the design, synthesis, and biochemical characterization of 30 non-nucleosidic quinazoline derivatives as METTL3/METTL14 inhibitors. The quinazoline scaffold mimics adenine interactions in the catalytic pocket and enables SAR exploration via substitutions at C2, C4, C6, and C7. Orthogonal biochemical assays identified sub-micromolar inhibitors. In HTRF assays, IC$_{50}$ values ranged from 120 to 740 nM, with **MC4774** as the most potent (119 nM). In the HotSpot radiometric assay ([^{3}H]-SAM incorporation), potency increased markedly: **MC4905** (1.56 nM), **MC4118** (1.86 nM), **MC4009** (2.15 nM), and **MC4774** (2.22 nM) (Figure 1). Compounds showed >1000-fold selectivity over EZH2, G9a, PRMT1, and SET8, with no inhibition up to 10 μM.

Cell assays showed a favorable therapeutic index: **MC4905** and **MC4009** preserved viability in CD34$^+$ cells (88–92%) and MCF10A cells (85–90%), while reducing MDA-MB-231 viability to ~25–30% at 5 μM (72 h), outperforming STM2457 [3]. Combination with cisplatin or olaparib enhanced antiproliferative effects. In MOLM-13 AML cells, IC$_{50}$ values were 1.47 and 1.59 μM, with 55–65% m6A reduction and ~65% c-MYC downregulation, confirming on-target activity.

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Macrocyclization as a Strategy for Optimizing Protacs Targeting Aurora Kinase A

Anouk Van Hauwermeiren[1], Simon Krols[1], Fien Martens[2], Hanne Van De Putte[1], Muhammad Rishifi[2], Kaat Durinck[2], Serge Van Calenbergh[1]

1. Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Belgium; Anouk.VanHauwermeiren@UGent.be
2. Department of Biomolecular Medicine, Faculty of Medicine & Health Sciences, Ghent University, Belgium

Keywords: *Aurora Kinase A, PROTACs, Macrocyclization, Neuroblastoma, Metabolic stability*

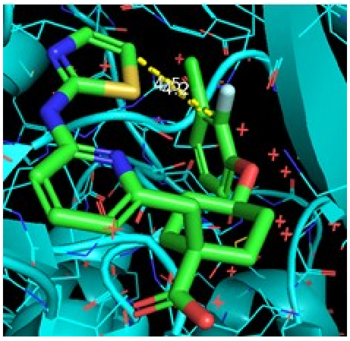

Figure 1. Co-crystal structure of MK-5108 bound to AURKA

Previous efforts at the Laboratory for Medicinal Chemistry led to the discovery of proteolysis-targeting chimeras (PROTACs) directed against Aurora kinase A (AURKA). Although these demonstrated potent in vitro degradation activity in neuroblastoma cell lines, their in vivo efficacy was limited, primarily due to poor metabolic stability, which hindered further development. [1] To address this limitation, macrocyclization was explored as a structural optimization strategy. By restricting conformational flexibility and limiting enzymatic accessibility, macrocyclization may improve the potency, selectivity, and metabolic stability of small molecules. [2–3] Guided by the co-crystal structure of the selective AURKA inhibitor MK-5108 bound to its target (figure 1), a series of macrocyclic AURKA ligands was designed, synthesized, and elaborated into AURKA-targeting PROTACs. Biological evaluation demonstrated that these macrocyclic PROTACs retained degradation potency comparable to their non-macrocyclic counterparts, indicating that macrocyclization is well tolerated within the PROTAC context. Notably, the macrocyclic PROTACs exhibited significantly improved metabolic stability, particularly with regard to cytochrome P450-mediated metabolism. Collectively, these findings highlight macrocyclization as a promising strategy to optimize PROTACs for improved pharmacokinetic properties.

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LIST OF ABSTRACTS

LSIT OF FLASH POSTER COMMUNICATIONS (FPCs)

Number	***Author***	***Title***
FPC1 *MORE*	*Pierfrancesco Cinque*	*Design and synthesis of molecular hybrids between drugs for dermatological disorders or wound healing and h2s donors*
FPC2 *MORE*	*Alberto Deplano*	*Selective activation of bacterial carbonic anhydrases as a strategy to modulate the gut microbiota*
FPC3 *MORE*	*Fabio Di Ricco*	*Targeting Nuclear Receptors for Therapeutic Modulation of Hepatic Lipid Accumulation via THR-β and PPARγ*
FPC4 *MORE*	*Stefano Giuffrida*	*New insights into PP structural features as class 3 CFTR correctors for combinatorial treatments in cystic fibrosis*
FPC5 *MORE*	*Giorgio Milli*	*Expanding the Chemical Space for LsrK Kinase Modulators: Hit Identification and Biological Validation for AI-2 Quorum-Sensing Quenching and Biofilm Formation Inhibition*
FPC6 *MORE*	*Angela Pagliaro*	*Structure-Based Design of RhoA Effector Interaction Inhibitors for the Treatment of Cancer*
FPC7 *MORE*	*Sara Salvi*	*Dimeric Sigma Receptor-Proteasome Ligands as Anticancer Agents for Glioblastoma*
FPC8 *MORE*	*Melania Elettra Vaccari*	*Evaluation of the Anticancer Activity of the Pan-Sigma Modulator RC-106 in a 3D Glioblastoma Model*
FPC9 *MORE*	*Sofia Vernile*	*Benzothiophene-based monoamine oxidase inhibitors: from design to biological evaluation*
FPC10 *MORE*	*Oriana Vitale*	*Comparative Study between Cholesterol and its Natural Derivative on RNA-lipid Interactions and Membrane Dynamics in Self-Assembling Lipid Nanoparticles*
FPC11 *MORE*	*Elif Yardimci*	*Novel N-Phenylacetamide Derivatives: In Vitro and In Silico Evaluation Against Prostate Cancer*

Design and synthesis of molecular hybrids between drugs for dermatological disorders or wound healing and H_2S donors

Pierfrancesco Cinque[1], Giuseppe Caliendo[1], Vincenzo Santagada[1], Rosa Sparaco[1], Vincenzo Calderone[2], Valentina Citi[2], Angela Ianaro[1], Giuseppe Ercolano[1], Barbara Maglione[3] and Francesco Frecentese[1]

1. Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy
2. Department of Pharmacy, University of Pisa, Via Bonanno, 6,56126, Pisa, Italy
3. Farmaceutici Damor Spa, Via Emilio Scaglione, 27, 80145 Napoli, Italy

pierfrancesco.cinque@unina.it

Keywords: *JAK-STAT pathway, wound healing, H_2S-donors*

The research project focuses on the development of molecular hybrids combining JAK kinase inhibitors, approved for dermatological diseases such as atopic dermatitis, psoriasis and vitiligo, with H_2S-releasing moieties able to mimic physiological hydrogen sulfide release [1]. These diseases are characterized by abnormal immune activation and chronic inflammation, with periods of clinical stability alternating with flare-ups.

In this study, molecular hybrids based on JAK-STAT pathway inhibitors, such as Abrocitinib and Peficitinib, were designed and synthesized by conjugating the JAK-inhibiting pharmacophore with selected H_2S donors, namely TBZ, ADT-OH and HBTA. This strategy aims to combine the anti-inflammatory activity mediated by JAK-STAT pathway inhibition with the well-known biological properties of H_2S [2]. TBZ is commercially available, whereas ADT-OH and HBTA were prepared according to published procedures [3,4]. Compounds I–VI were evaluated for cytotoxicity in HaCaT keratinocytes at different concentrations ranging from 0.01 to 100 μM. Their H_2S-releasing profile was also assessed in the presence and absence of L-cysteine. In vitro pharmacological studies were performed at 5 μM, including scratch test assays to evaluate their wound healing potential. Overall, compounds I–VI showed promising results, with HBTA and ADT-OH derivatives, particularly compounds II, III, V and VI, displaying the best activity and promoting complete wound closure within 48–72 h. Further studies are ongoing to assess their anti-inflammatory activity and to confirm JAK-STAT pathway inhibition through molecular biology assays.

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Selective activation of bacterial carbonic anhydrases as a strategy to modulate the gut microbiota

Deplano Alberto[1], Moi Davide[1], Carradori Simone[2], Gallorini Marialucia[2], Angeli Andrea[3], Iacovozzi Damiano[2], Onnis Valentina[1], Capasso Clemente[4], Supuran Claudiu Trandafir[3].

1. Department of Life and Environmental Sciences, University of Cagliari, University Campus, S.P. 8 CA, 09042 Monserrato, Italy
2. Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy
3. NEUROFARBA Department, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, FI, Italy
4. Department of Biology, Agriculture and Food Sciences, National Research Council, Institute of Biosciences & Bioresources, Naples, 80131, Italy

alberto.deplano@unica.it

Keywords: *Carbonic anhydrase, Activators, Dysbiosis.*

The gut microbiota is a community of microorganisms that live in the digestive tracts of humans and animals [1]. It plays an important role in many physiological functions, including protection against pathogens, digestion, enhancement of the immune system, and the regulation of epithelial cell proliferation and differentiation. The alteration of the microbial composition, known as dysbiosis, can lead to the colonization of resistant pathogens and the overgrowth of opportunistic species and is associated with the development of several diseases. The most commonly employed approach to restore the microbiota is the administration of probiotics, which can inhibit the colonization of pathogenic bacteria, being Bifidobacteria and Lactobacilli the most extensively used. In this context, the development of small molecules capable of modulating the activity of key bacterial enzymes may represent an effective strategy for the treatment of dysbiosis. A promising strategy may involve enhancing their enzymatic activity through the selective activation of carbonic anhydrases in these probiotic strains. This hypothesis remains largely unexplored due to the limited scientific literature currently available in this field. In contrast, while structure-activity relationships for the development of carbonic anhydrase inhibitors have been extensively studied and are well established, the selective activation of these enzymes remains considerably more challenging and significantly less explored [2]. Herein we describe the design, synthesis, and evaluation of a novel class of bacterial carbonic anhydrase activators based on biogenic amines, amino acids, and short peptides, all of which are established carbonic anhydrase activators. The synthesized compounds were found to activate bacterial carbonic anhydrases with K_a values in the low micromolar range. Importantly, they showed no detectable activity against human carbonic anhydrases, indicating a high degree of selectivity. The most promising derivatives were selected for further biological evaluation.

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Targeting Nuclear Receptors for Therapeutic Modulation of Hepatic Lipid Accumulation via THR-β and PPARγ

Di Ricco Fabio[1], Caddeo Andrea[2], Kowalik Marta Anna[2], Perra Andrea[2], Manera Clementina[1], Rapposelli Simona[1]

1. Department of Pharmacy, University of Pisa, Via Bonanno Pisano 6, 56126 Pisa, Italy;
2. Department of Biomedical Sciences, University of Cagliari, Unit of Oncology and Molecular Pathology, 09042 Monserrato, Italy.

fabio.diricco@phd.unipi.it

Keywords: *MASLD, MASH, HCC, THR-β, PPARγ*

Metabolic dysfunction is a major driver of liver diseases, including metabolic dysfunction–associated steatotic liver disease (MASLD), its progressive form metabolic dysfunction–associated steatohepatitis (MASH), and ultimately hepatocellular carcinoma (HCC). Due to the multifactorial nature of these conditions, nuclear receptor modulation has emerged as a promising therapeutic strategy, given its central role in regulating metabolic homeostasis. Among these targets, thyroid hormone receptor beta (THR-β) and peroxisome proliferator-activated receptor gamma (PPARγ) have gained increasing attention. THR-β agonists enhance hepatic lipid metabolism, while PPARγ, upon heterodimerization with retinoid X receptor alpha (RXRα), regulates adipogenesis and lipid storage. In this study, we investigated the effects of two compounds acting on these pathways: TG68, a prodrug targeting THR-β **[1]**, with IS25 as its bioactive form, and BVT.13, a PPARγ modulator **[2]** (**Figure 1**). Cell viability assays in HepG2 cells demonstrated that both compounds are well tolerated, with no significant cytotoxicity at the tested concentrations. We then evaluated their impact on intracellular lipid accumulation, a hallmark of MASLD. Treatment with either TG68 or BVT.13 significantly reduced intracellular lipid content, indicating a beneficial effect on hepatic lipid metabolism. Given their complementary mechanisms of action, we further explored the potential synergistic effects of combined treatment. Based on these findings, a structure-activity relationship (SAR) study was initiated to design and synthesis new analogues of BVT.13 and TG68 in order to further investigate them alone and/or in combination as a potential treatment to counteract MASLD.

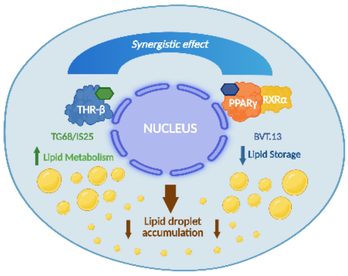

Figure 1. Synergistic targeting of THR-β and PPARγ in hepatic lipid metabolism

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New insights into PP structural features as class 3 CFTR correctors for combinatorial treatments in cystic fibrosis

Stefano Giuffrida[1], Marilia Barreca[1], Mario Renda[2], Fabiana Lo Mascolo[1], Alessandra Lipani[2], Roberta Bivacqua[1], Daniela Guidone[2], Michele Genovese[2], Maria Valeria Raimondi[1], Virginia Spanò[1], Alessandra Montalbano[1], Fabio Bertozzi[3], Tiziano Bandiera[3], Francesco Ortuso,[4] Stefano Alcaro,[4] Luis Juan Vicente Galietta[2], and Paola Barraja[1]

1. University of Palermo, Department of biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo; Italy
2. Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA); Italy
3. Istituto Italiano di Tecnologia (IIT), Genova, Italy
4. Magna Graecia University, Department of Health Sciences, Catanzaro; Italy

stefano.giuffrida@unipa.it

Keywords: *Cystic fibrosis, CFTR, CFTR modulators, class 3 correctors*

Cystic fibrosis (CF) is a genetic disease caused by mutations that impair the function of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel. This defect can be overcome by CFTR "correctors", small molecule modulators that stabilize the misfolded protein and increase membrane expression [1-3]. Significant progresses have been made in the pharmacological treatment of CF with the development of Kaftrio, a triple combination of two correctors (VX-661, VX-445) and one potentiator (VX-770) and, recently, of Alyftrek, a combination of deutivacaftor, VX-661 and vanzacaftor [4]. However, the development of new CFTR modulators is an important goal to maximize mutant CFTR rescue. In the course of our studies, we have identified a new class of small molecules (called PP compounds) as correctors with high efficacy in the rescue of F508del-CFTR on native epithelial cells of CF patients, particularly in combination with class 1 correctors (VX-809) [5]. A multiparametric optimization of PP compounds is ongoing. In parallel, we are also investigating another family of correctors, called SH compounds, derived from PP compounds using a scaffold hopping approach. These compounds bearing a higher conformational flexibility and offering multiple regions for structural diversification allow the generation of a wide family of analogues with higher optimization potential. The new compounds are tested using the HS-YFP functional assay on CFBE41o- cells expressing F508del-CFTR mutation. The most active compounds are then validated: i) in short-circuit recordings on primary airway epithelial cells; ii) in biochemical assays to evaluate CFTR maturation/trafficking. So far, we have evaluated 450 compounds from which several effective derivatives emerged with EC_{50} values at sub-micromolar level. Mechanistic studies have shown that PP and SH compounds act as class 3 correctors, able to produce a strong synergistic/additive effect when combined with class 1 agents such as VX-809 and VX-661. Selected candidates were also evaluated for their *in vivo* pharmacokinetic profile in rodents to preliminary asses their drug-like profile. We have also tested the efficacy and potency of two selected compounds, PP028 and SH157A, when combined with another type of class 1 correctors [6]. In particular, combination of ARN22081, ARN22151, or ARN22361 class 1 correctors with single digit nanomolar potency with PP028 or SH157A markedly enhanced F508del-CFTR rescue. Interestingly, the use of PP028/SH157A produced an increase of the potency of ARN correctors, with a shift of the EC_{50} values to the sub-nanomolar range. These results confirm the ability of the PP and SH compounds to synergize with ARN correctors thus indicating the possibility to develop novel pharmacological combinations to rescue mutant CFTR.

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Identification and Biological Validation for AI-2 Quorum-Sensing Quenching and Biofilm Formation Inhibition

Milli Giorgio[1], Ambrosio Francesca Alessandra[2], Bellan Menegussi Elisa[3], Pagano Katiuscia[4], Ragona Laura[4], Costa Giosuè[2], Alcaro Stefano[2], Pietrocola Giampiero[3], Linciano Pasquale[1], Collina Simona[1]

1. Department of Drug Sciences, University of Pavia, Viale Taramelli 12, Pavia 27100, Italy
2. Department of Health Sciences, University "Magna Græcia" of Catanzaro, Viale Europa, 88100 Catanzaro, Italy
3. Department of Molecular Medicine, Biochemistry Unit, University of Pavia, Viale Taramelli 3/b, Pavia 27100, Italy
4. NMR Laboratory, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", CNR, via Alfonso Corti, 12, Milano 20133, Italy

giorgio.milli01@universitadiapavia.it

Keywords: *Antimicrobial resistance, Quorum Sensing, Biofilm,* LsrK inhibiotrs.

Antimicrobial resistance (AMR) poses a critical global health crisis by undermining existing treatments and threatening public health. Consequently, identifying novel targets and antimicrobials with innovative mechanisms of action has become essential to combat AMR [1]. In this context, disrupting quorum sensing (QS) and biofilm formation emerges as a promising strategy. LsrK, a kinase that initiates QS and promotes biofilm development through phosphorylation of 4,5-dihydroxy-2,3-pentanedione (DPD) - a ubiquitous signaling molecule in both Gram-positive and Gram-negative bacteria - represents a critical component of QS pathways and its inhibition could be a winning strategy for fighting AMR [2].

To this end, structure-based virtual screening of commercial libraries identified a panel of hit molecules, which were evaluated in biological, following an experimental pathway previously optimized [3], to confirm their ability to inhibit biofilm formation in three bacterial models: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. We developed a dedicated synthesis strategy to obtain sufficient quantities of the most promising compounds for comprehensive biological and microbiological evaluations. Their ability to interfere with quorum sensing was assessed in *Vibrio harveyi* reporter assays, while interaction with LsrK was confirmed by biophysical studies, including Saturation Transfer Difference (STD) NMR.

These efforts identified two lead compounds, CzdDPD 8 and CzDPD 10, with a Minimum Biofilm Inhibitory Concentration 50% ($MBIC_{50}$) values of 121 and 183 $\mu g\ mL^{-1}$ against *E. coli*, and 55 and 166 $\mu g\ mL^{-1}$ against *P. aeruginosa*, respectively. Mechanistic studies validated QS disruption and LsrK binding, marking a significant advance against AMR.

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Structure-Based Design of RhoA Effector Interaction Inhibitors for the Treatment of Cancer

Pagliaro Angela[1,2], Brindani Nicoletta[1], Bergamaschi Enrico[1], Vitali Forconesi Gabriella[1], Maria Antonietta La Serra[1], Veronesi Marina[1], Cardoso De Azevedo Moreira Miguel Angelo[1], Bonaldo Valerio[1], Girotto Stefania[1], De Vivo Marco[1]

1. Italian Institute of Technology (IIT), Genova, Italy;
2. Department of Pharmacy and Biotechnology Fabit, University of Bologna, Bologna, Italy;

angela.pagliaro@iit.it

Keywords: *RhoA, ROCK1, Anticancer, PPI,* and *Organic synthesis*

Rho GTPases are a family of small G proteins that regulate essential physiological processes, including cytoskeletal dynamics and cell cycle progression. Our research laboratory has been actively involved in the field of protein-protein Interactions. Previous studies focused on disrupting the complex between the GTPase CDC42 and its effector PAK protein, leading to the identification of lead compounds with anticancer activity in vivo. These have shown a good pharmacokinetic profile, bioavailability, and efficacy in multiple in vivo PDX tumor mouse models [1]. Building on these results, we have now turned our focus toward inhibiting the RhoA/ROCK1 interaction. This plays a pivotal role in cancer pathology, with RhoA being overexpressed or hyperactivated in several tumors. In melanoma, the activation of RhoA and its downstream kinase, ROCK1, is directly correlated with disease progression through deregulation of cell motility [2,3]. We started with a virtual screening and identified twenty candidate compounds. These underwent an intrinsic fluorescence assay to test their binding with RhoA. Three compounds were identified as promising hits. Synthesis of a few analogs confirmed their activity and a favorable DMPK profile. Based on these preliminary data, we are currently pursuing further optimization of these promising compounds to block RhoA.

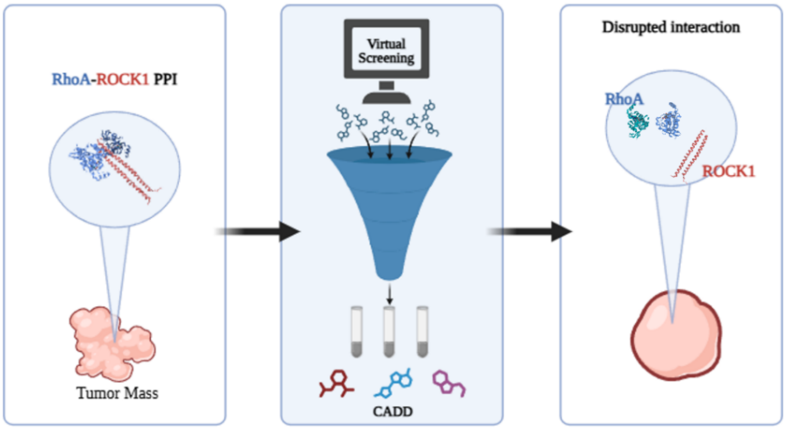

Figure 1. Project Workflow

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Dimeric Sigma Receptor-Proteasome Ligands as Anticancer Agents for Glioblastoma

Salvi Sara[1], Rossino Giacomo[1], Vaccari Melania Elettra[1], Malacrida Alessio[2], Molteni Laura[2], Miloso Mariarosaria[2], Collina Simona[1]

1. Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia (PV), Italy
2. Department of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900, Monza (MB), Italy

sara.salvi01@universitadipavia.it

Keywords: *glioblastoma, anticancer, Sigma receptors, proteasome, multitarget*

Glioblastoma multiforme (GBM) is one of the most aggressive and lethal brain tumors, accounting for more than half of all malignant brain tumors [1]. This work aims to identify a new generation of anticancer agents for GBM treatment through a multitarget-directed ligand (MTDL) approach, simultaneously targeting Sigma receptors (SRs) and the proteasome, whose interconnected roles support their combined modulation. In previous studies, we identified two hit compounds targeting SRs and the proteasome, respectively: **RC106** and **IP24** (Figure 1). Both compounds exhibit antiproliferative activity against various cancer cell lines [2,3].

In the present study, these two scaffolds were covalently combined to generate a small set of dimeric MTDLs. **RC106** and **IP24** were linked through different spacers to achieve dual SR-proteasome modulation and improved antitumor efficacy. A total of four dimeric compounds were synthesized, exploring the effect of different derivatization strategies and inter-pharmacophore distance.

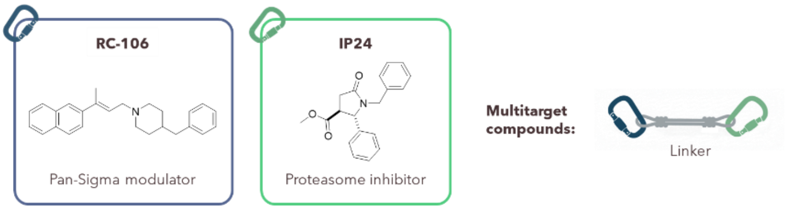

Figure 1. Chemical structure of **RC106** and **IP24**, and schematic representation of final dimeric compounds.

To assess whether dimeric architecture could compromise target engagement, the conformation of a representative compound was investigated in aqueous solution using one- and two-dimensional NMR experiments. The experimental data, supported by computational studies, showed that the dimer adopts an extended conformation, which is crucial for effective dual target interaction.

Biological evaluation is currently ongoing, and includes antiproliferative assays on GBM cell lines, as well as the assessment of SRs binding affinity and proteasome inhibition, in order to confirm the proposed multitarget mechanism of action.

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Evaluation of the Anticancer Activity of the Pan-Sigma Modulator RC-106 in a 3D Glioblastoma Model

Vaccari Melania E.[1], Salvi Sara[1],Tosca Elena[2], Aiello Ludovica[2], Magni Paolo[2] and Collina Simona [1]

1. Department of Drug Sciences, MedChemLab, University of Pavia, Pavia, Italy
2. Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy

melaniaelettra.vaccari01@univeristadipavia.it

Keywords: *sigma receptors,cancer, glioblastoma, 3D*

Sigma receptors comprise two subtypes, namely Sigma-1 (S1R) and Sigma-2 (S2R) receptors, which are involved in a variety of biological processes. S1R agonists have been associated with neuroprotective effects, whereas S1R antagonists have shown promising potential in cancer treatment. S2R agonists have demonstrated anticancer activity across different tumor models[1–2]. The story of MedChemLab is closely linked to SRs, and led to the identification of several novel SRs ligands. Among these, RC-106 emerged as a pan-sigma modulator with promising anticancer activity[3-4]. Specifically, RC-106 exhibits a dual pharmacological profile, acting as an S1R antagonist and an S2R agonist, and has demonstrated cytotoxic activity in three pancreatic cancer cell lines (PANC-1, Capan-1, and Capan-2), all of which express SRs. Moreover, RC-106 disrupted PANC-1 spheroids within 48 hours of treatment and induced cell death in all cell lines tested[3]. Encouraged by these promising findings, we investigated the potential anticancer activity of RC-106 in the glioblastoma (GBM) cell line U87. GBM represents the most aggressive primary brain tumor in adults, with a median survival of 14 months, due to therapeutic resistance and limited efficacy of current treatment options[4]. In this context, RC-106 was first evaluated in 2D cultures to assess its cytotoxic effects, and subsequently in a 3D model to better mimic tumor architecture and microenvironmental conditions. These experiments aimed to determine RC-106 ability to inhibit spheroid growth and to promote spheroid disaggregation, thereby providing further insight into its therapeutic potential in GBM. Moreover, to gain deeper insight into the internal structure of the spheroids beyond size measurements, spheroids were embedded in paraffin and sectioned to obtain histological slices. These sections were stained using the hematoxylin and eosin technique to evaluate cell viability and density within the spheroid core. These data further confirm the anticancer activity of RC-106 and will contribute to the development of a mathematical model aimed at predicting the in vivo efficacy of the compound.

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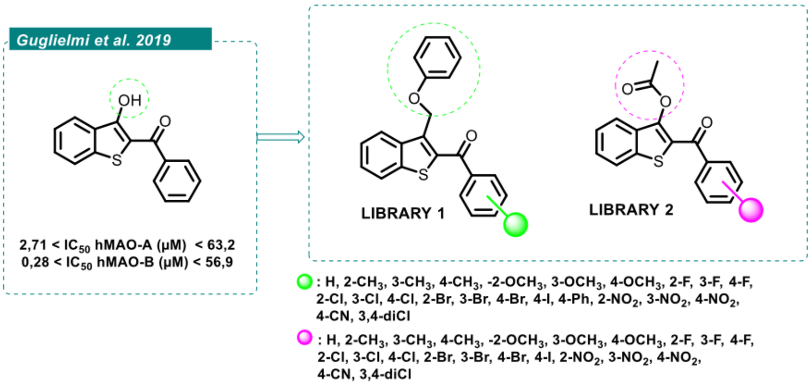
Benzothiophene-based monoamine oxidase inhibitors: from design to biological evaluation

Sofia Vernile[1], Francesca Arrighi[1], Michele Coluccia[1], Arianna Granese[1], Paola Chimenti[1], Daniela Secci[1], Paolo Guglielmi[1]
1. Dept of Chemistry and Technologies of Drugs, Sapienza University of Rome, Rome, Italy;

sofia.vernile@uniroma1.it

Human monoamine oxidases (hMAOs) are mitochondrial flavoenzymes that break down amines and regulate neurotransmitters such as serotonin, dopamine, and norepinephrine. Two isoforms have been identified in humans, namely hMAO-A and hMAO-B, which differ in their activity, tissue distribution and substrate specificity. The selective inhibition of the B isoform represents a kay strategy in the management of neurodegenerative disorders, including Parkinson's disease[1]. Previous studies showed that benzo[*b*]thiophen-3-ol derivatives act as selective hMAO-B inhibitors, exhibiting neuroprotective profile in ex-vivo models[2]. With the aim of investigating the potential of this scaffold, two series of benzo[*b*]thiophen-3-ol derivatives, benzylated (Library 1, **Figure 1**) and acetylated (Library 2, **Figure 1**), were designed and evaluated for their inhibitory activity in this study. Acetylated compounds were generally strong hMAO inhibitors but lacked selectivity, affecting both hMAO-A and hMAO-B. In contrast, benzylated compounds showed activity and selectivity depending on the type and position of substituents on the aromatic ring. These findings highlight the critical role of structural modifications in modulating isoform selectivity and support the further optimization of benzylated derivatives as promising hMAOs inhibitors.



● : H, $2\text{-}CH_3$, $3\text{-}CH_3$, $4\text{-}CH_3$, $\text{-}2\text{-}OCH_3$, $3\text{-}OCH_3$, $4\text{-}OCH_3$, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 4-I, 4-Ph, $2\text{-}NO_2$, $3\text{-}NO_2$, $4\text{-}NO_2$, 4-CN, 3,4-diCl

● : H, $2\text{-}CH_3$, $3\text{-}CH_3$, $4\text{-}CH_3$, $\text{-}2\text{-}OCH_3$, $3\text{-}OCH_3$, $4\text{-}OCH_3$, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 4-I, $2\text{-}NO_2$, $3\text{-}NO_2$, $4\text{-}NO_2$, 4-CN, 3,4-diCl

Figure 1. Design of the novel derivates.

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Comparative Study between Cholesterol and its Natural Derivative on RNA-lipid Interactions and Membrane Dynamics in Self-Assembling Lipid Nanoparticles

Oriana Vitale[1], Antonio De Nicola[2], Valeria Nele[1], Giuseppe De Rosa[1], Bruno Catalanotti[1] and Federica Moraca[1]

1. University of Naples "Federico II", Department of Pharmacy, via Domenico Montesano, 49, 80131 Naples, Italy.
2. Department of High-Performance Computing, CINECA, Casalecchio di Reno, 40033 Bologna, Italy.

oriana.vitale@unina.it

Keywords: *Self-assembling nanoparticles, Molecular Dynamics, Cholesterol, RNA.*

Self-assembling nanoparticles (SANPs) are lipid-based nanocarriers composed of a lipid bilayer formed by cationic lipids, sterols, and PEGylated lipids, enclosing a calcium phosphate core capable of encapsulating RNA, demonstrating high efficacy as nucleic acid delivery platforms, particularly in the treatment of glioblastoma [1]. SANPs are typically formed under basic conditions (pH ~9) through a self-assembly process triggered by mixing lipid components with calcium phosphate and ncRNA, leading to the spontaneous formation of organized nanoscale structures. A distinctive feature of these nanoparticles is their organization, often described as micellar or core-shell, which contributes to their stability and encapsulation efficiency [1]. In this context, the role of sterols is crucial. Cholesterol, commonly used in lipid formulations, is known to increase bilayer order and rigidity, thereby enhancing the structural stability of nanoparticles. Cholesterol derivatives, characterized by a bulkier side chain, can alter lipid packing, membrane fluidity, and curvature, thus influencing lateral domain formation and potentially improving RNA encapsulation efficiency [2][3]. To investigate these effects, atomistic molecular dynamics simulations (AA-MD) have been performed on systems containing cholesterol and its natural derivative, both in the presence and absence of RNA. Parameters such as lipid lateral diffusion, order parameters, density profiles, RNA solvent-accessible surface area (SASA), and RNA-lipid distances were analyzed to elucidate the role of sterols in complex stabilization and in the mechanisms governing RNA-membrane interactions [4]

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Novel *N*-Phenylacetamide Derivatives: *In Vitro* and *In Silico* Evaluation Against Prostate Cancer

Yardimci Elif [1,2], Erzurumlu Yalcin [3], and Yildiz Ilkay [4]

1. Ankara University, Graduate School of Health Science, Ankara, Türkiye
2. Agri Ibrahim Cecen University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Agri, Türkiye
3. Süleyman Demirel University, Faculty of Pharmacy, Department of Biochemistry, Isparta, Türkiye
4. Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Türkiye

eyardimci@agri.edu.tr

Keywords: *N-phenylacetamide, prostate cancer, antiproliferative activity, molecular docking, ADME*

Prostate cancer is among the most prevalent malignancies in men worldwide and remains a significant challenge in cancer therapy. Although numerous therapeutic options and drugs are currently used, the development of drug-resistant cancer cells and limited clinical efficacy highlight the need for new alternative therapeutic strategies [1]. *N*-phenylacetamide is a scaffold associated with a wide range of biological activities, including anticancer, antifungal, antibacterial, antileishmanial, antidepressant, anticonvulsant, antimalarial, antiviral, analgesic, and antipyretic activities [2]. Moreover, numerous anticancer drugs containing an amide moiety in their structures are currently used in cancer therapy. In this study, a series of novel *N*-(2-hydroxy-4-chlorophenyl)acetamide derivatives were designed and synthesized [3] as potential anticancer agents and evaluated for the cytotoxicity against non-neoplastic (PNT1A) and neoplastic (LNCaP and DU145) human prostate cell lines using colorimetric-based WST-1 cell viability test [4], with etoposide serving as the reference drug. To investigate the molecular basis of these effects, *in silico* studies including molecular docking and ADME predictions were performed using Cresset Flare. The *N*-phenylacetamide derivatives were docked into the androgen receptor obtained from the Protein Data Bank (PDB:5V8Q) to assess binding interactions, while computational ADME analysis provided insights into their pharmacokinetic properties and drug-likeness. *In vitro* studies of the synthesized compounds demonstrated significant anticancer activity against prostate cancer. Furthermore, molecular docking studies revealed binding energies consistent with the *in vitro* results. In conclusion, these *N*-phenylacetamide derivatives may represent promising candidates for the treatment of prostate cancer.

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PE-MEDCHEM EURO PHD LABEL COMMUNICATIONS

Number	Author	Title
PEAward1	*Giorgio Buttitta*	*Liposomes: the gold standard in drug delivery technology*
PEAward2	*Laura Cantudo*	*Computer-aided drug design and evaluation of enzyme inhibitors, PROTACs and LYTACs targeting cancer and other diseases*
PEAward3	*Adriana Gargano*	*Discovery and Characterization of Natural Compounds for Cancer Therapy: A Combined Target-Based and Phenotypic Approach*
PEAward4	*Ihor Yushyn*	*Synthesis and biological activity of thiazolidine derivatives with a pyrazoline fragment in molecules*
PEAward5	*Emanuela Marchese*	*In Silico Approaches to Uncover the Antioxidant and Therapeutic Properties of Polyphenols*
PEAward6	*Barbara Nunes*	*Quaternary ammonium and phosphonium salts as antimicrobial agents: synthesis, biological evaluation, and mechanistic insights*
PEAward7	*Martina Piga*	*Targeting NaV and HV1 channels: discovery of novel small-molecule inhibitors*
PEAward8	*Miguel Pinto*	*Using a nanomedicine-based approach as a second chance for tolcapone*
PEAward9	*Lorenzo Raffellini*	*Targeting autophagy through ampk: design, synthesis, and biological evaluation of novel allosteric modulators*
PEAward10	*Benedetta Salvucci*	*Development of Novel Antimalarial Transmission Blocking Agents and Genetic Characterization of Drug Resistance in Mycobacterium abscessus*

Liposomes: the gold standard in drug delivery technology

Buttitta Giorgio[1], Luciani Paola[2], Bonacorsi Simone[1,3], Barbarito Chiara[3], Moliterno Mauro[3], Pompei Simona[3], Saito Gabriele[3], Oddone Irene[3], Verdone Giuliana[3], Raimondi Sergio[3], Guglielmi Paolo[1] and Secci Daniela[1]

1. Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
2. Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland
3. BSP Pharmaceuticals S.p.A., Via Appia Km. 65,561, 04013 Latina Scalo (LT), Italy.

giorgio.buttitta@uniroma1.it

Liposomes are phospholipid vesicles able to encapsulate hydrophilic and hydrophobic compounds, enabling controlled drug delivery with high biocompatibility and clinical relevance. This industrial PhD thesis investigates advanced liposomal drug delivery systems through three interconnected projects focused on sustained release, formulation optimization, and scalable manufacturing. The first project, conducted at the University of Bern, addressed the limitations of carprofen, a poorly soluble nonsteroidal anti-inflammatory drug used in veterinary medicine. A depot liposomal formulation was developed to achieve prolonged release after subcutaneous administration. Formulation screening and physicochemical characterization enabled optimization of the system, while an agarose gel matrix model simulating the subcutaneous environment was designed to evaluate sustained-release behavior in vitro. Preliminary scale-up demonstrated translational potential. The second project focused on lipid-based carriers for neurodegenerative disease treatment. A human monoamine oxidase B inhibitor was incorporated into highly PEGylated liposomes. Using Design of Experiments combined with microfluidics, critical process parameters were optimized to obtain formulations with controlled quality attributes, demonstrating the value of integrating medicinal chemistry, nanotechnology, and formulation science. The final project, carried out at BSP Pharmaceuticals, established a scalable microfluidic platform for liposome manufacturing. Commercial-like formulations inspired by Doxil® and Marqibo® were reproduced and optimized, generating highly monodisperse and regulatory-compliant liposomes. The platform enabled rapid scale-up, producing up to 1 L of formulation in less than 20 min. Overall, this thesis demonstrates how rational formulation design, microfluidic technologies, and scalable manufacturing can advance liposomal nanomedicine from laboratory research to industrial production.

Computer-aided drug design and evaluation of enzyme inhibitors, PROTACs and LYTACs targeting cancer and other diseases

Laura Márquez-Cantudo[1]

1. .Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, 28668, Spain.

laura.marquezcantudo@ceu.es

The work combines classical and advanced tools in computational chemistry and molecular modelling for the design and computational evaluation of enzyme inhibitors (traditional and dual inhibitors), PROTACs (PROteolysis TArgeting Chimeras), and LYTACs (LYsosome TArgeting Chimeras), as new therapeutic strategies for the treatment of cancer, osteoarthritis, and amebiasis. [1]. Osteoarthritis is a degenerative joint condition that is characterized by pain, swelling, and stiffness, thereby abruptly impacting the quality of life of mainly elderly people. Future projections to 2060 indicate that around 765 million individuals worldwide will suffer from this life-impairing pathology. Our approach relies on inhibiting Matrix Metalloproteinase 13 (MMP-13) as this enzyme is known to be overexpressed in this disease and contributes to joint degeneration by degrading collagen type II. For this, molecular docking and ADMET property prediction strategies were used to design a new promising series of inhibitors and to evaluate their druggability [2]. *Acanthamoeba castellanii* is a free-living amoeba capable of causing keratitis in healthy individuals and fatal granulomatous encephalitis in immunocompromised patients. During its life cycle, the organism can transition from the trophozoite (infective form) to the cyst (resistant form), the latter being particularly difficult to eradicate. A leucine aminopeptidase belonging to the M17 superfamily (AcLAP) has been identified as crucial in the encystment process. In this context, inhibition of AcLAP is proposed as a promising therapeutic strategy against this amoeba. For that, four compounds designed and synthesized in our research group were repurposed and evaluated as potential inhibitors using molecular dynamics simulations. Additionally, an alternative catalytic mechanism for this enzyme is proposed based on hybrid QM/MM methods. Cancer is a multifactorial and highly complex pathology that is expected to affect more than 2 million people in 2026. Three different strategies were explored in this work as possible cancer treatments: a) dual inhibition of Protein Kinase 2 (CK2) and Histone Deacetylases (HDACs); b) CK2-targeting PROTACs, and c) MMP-2-directed LYTACs [3]. The deregulation of these three enzymes has been associated with the proliferation, migration, and survival of tumour cells, making them attractive therapeutic targets for this disease. The binding mode of new series of these compounds was assessed by means of molecular docking and molecular dynamics simulations. In addition, to predict the permeability of the CK2-directed PROTACs, two different approaches were followed: conformational search using a Monte Carlo-combined algorithm and umbrella sampling simulations to assess their passive diffusion mechanism through plasmatic membranes.

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Discovery and Characterization of Natural Compounds for Cancer Therapy: A Combined Target-Based and Phenotypic Approach

Gargano Adriana[1,2], Puerta Adrián[3], González Bakker Aday[3], Rocca Roberta[1], Maggisano Valentina[1], Bulotta Stefania[1], Padrón José M.[3] and Alcaro Stefano[1,2]

1. Dipartimento di Scienze della Salute, Università “Magna Græcia” di Catanzaro, Campus Universitario “S. Venuta”, Viale Europa, 88100 Catanzaro, Italy;
2. Associazione CRISEA – Centro di Ricerca e Servizi Avanzati per l’Innovazione Rurale, Località Condoleo, 88055 Belcastro (CZ), Italy;
3. BioLab, Instituto Universitario de Bio-Orgánica “Antonio González”, Universidad de La Laguna, C/ Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain;

a.gargano@unicz.it

Keywords: *Natural Compounds, Drug Discovery, Molecular Modeling, Anticancer Activity, Mediterranean Area.*

This PhD project identifies natural Mediterranean compounds endowed with pharmaceutical activity using both target-based drug discovery and phenotypic drug discovery approaches across five cancer-focused studies. The first project involved the synthesis of coumarin-lipophilic cation hybrids as selective inhibitors of mitochondrial Carbonic Anhydrases IX and XII. These showed potent, selective activity against various cancer cells, including multidrug-resistant models, via a "suicide inhibition" mechanism; phosphonium salts exert cytostatic effects, whereas guanidinium derivatives trigger apoptosis [1]. The second project investigated the modulation of the IL-20RA receptor, through a virtual screening of natural compounds and FDA-approved drugs, Ritonavir was identified as a potential inhibitor. Biological assays confirmed its dose-dependent efficacy, validated by IL-20RA silencing. [2]. The third and fourth projects targeted HDAC8 and Sphingosine Kinase 1 (SK1), respectively. Shape-based virtual screening identified a potent HDAC8 inhibitor that demonstrated high enzymatic inhibition (97.56% at 1 μM) and induced apoptosis in multiple cancer cell lines. For SK1, a pharmacophore-based screening identified 7 candidate ligands, currently undergoing enzymatic evaluation. Finally, the fifth project utilized a PDD approach to study two novel compounds from Argentinean Maytenus species: 20-α-hydroxyscutione and 15-α-hydroxytingenone [3]. Both compounds exhibited anti-migratory and pro-apoptotic effects on lung cancer cells without disrupting microtubule dynamics. Structural similarity searches identified related compounds in Mediterranean plants, such as Taraxacum officinale and Bituminaria bituminosa, providing a scientific basis for the design of a specialized botanical garden in collaboration with regional research centers. Overall, this work integrates computational modeling and biological validation to discover new therapeutic leads and supports the valorisation of Mediterranean botanical resources in drug discovery.

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Synthesis and biological activity of thiazolidine derivatives with a pyrazoline fragment in molecules

Yushyn Ihor[1]
1. Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, 69 Pekarska St., 79010 Lviv, Ukraine

Pyrazoline–thiazolidine hybrids are considered promising scaffolds because of their ability to interact with multiple biologically relevant targets, including kinases, inflammatory mediators, and nuclear receptors, which determines their broad pharmacological potential [1,2]. The dissertation focused on the synthesis of novel pyrazoline-containing thiazolidine and related derivatives as promising bioactive heterocycles. Efficient synthetic approaches, including [2+3]-cyclocondensation, Knoevenagel condensation, Claisen–Schmidt reaction, and hetero-Diels–Alder reaction, enabled the preparation of structurally diverse pyrazolyl-substituted 4-thiazolidinones, thiazoles, and fused heterocyclic systems. Pyrazole-derived carbothioamides proved to be effective S,N-binucleophilic reagents for obtaining new thiazolidinone derivatives, while reactions of isorhodanine with 3-methyl-1H-pyrazol-5-amine afforded key intermediates for further functionalization. Unexpected transformations led to the formation of condensed pyrazolo[3,4-b]thiazolo[5,4-e]pyridines. Structures were confirmed by LC–MS, NMR, and X-ray analysis. Biological screening identified compounds with antitumor, antimicrobial, antifungal, and anti-inflammatory activities; some active derivatives also suggested possible PARP1/2 inhibition. The most active compounds showed selective cytotoxicity toward Jurkat and MCF-7 cell lines, while selected fused heterocycles demonstrated activity against Gram-positive microorganisms.
Overall, 70 compounds were synthesized, and 20 hit compounds were identified.

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In Silico Approaches to Uncover the Antioxidant and Therapeutic Properties of Polyphenols

Marchese Emanuela[1], Costa Giosuè[1], and Alcaro Stefano[1,2]

1. Dipartimento di Scienze della Salute, Università“Magna Græcia” di Catanzaro, Campus “S. Venuta”, 88100, Catanzaro, Italy.
2. Associazione CRISEA-Centro di Ricerca e Servizi Avanzati per l’innovazione Rurale, Loc. Condoleo, Belcastro 88055, Italy

e.marchese@unicz.it

Keywords: *polyphenols*, *molecular modeling*, *antioxidant mechanisms*, and *drug repurposing*

Natural products represent a key component in drug discovery, providing structurally diverse bioactive compounds with significant therapeutic potential. An integrated in silico and experimental approach was employed to elucidate both the antioxidant and target-mediated biological properties of polyphenols and their metabolites. Density Functional Theory calculations were used to investigate the radical scavenging mechanisms of gut microbiota-derived metabolites of ellagic acid. Thermodynamic and kinetic analyses identified urolithin D as the most effective compound in neutralizing hydroperoxyl radicals in aqueous environments. Urolithins A and C also demonstrated antioxidant potential, whereas urolithin B showed negligible activity. Experimental assays confirmed that antioxidant activity predominantly proceeds via a single electron transfer (SET) mechanism [1]. The same computational approach was applied to the flavonoid (+)-catechin, highlighting its ability to neutralize free radicals through a hydrogen atom transfer (HAT) mechanism [2]. Beyond the renowned antioxidant properties of polyphenols, the research was turned to investigate target-mediated effects. A virtual screening protocol was able to suggest two best candidates, diosmin and hesperidin, from a subset of FDA-approved polyphenols, as promising proteasome inhibitors. Subsequent biological evaluations confirmed their capacity to inhibit proteasome activity and exert anti-tumor effects in both sensitive and resistant cancer cell lines [3]. Finally, the interaction of urolithins with presynaptic α_2-adrenergic receptors was investigated through molecular docking and molecular dynamics simulations. Computational results were fundamental to disclose a huge heterogeneity in the modes by which the three different metabolites interact with the α_2 receptor, providing a molecular-level interpretation of the experimental functional data [4].

These findings highlight the value of combining computational and experimental approaches to unravel the mechanisms of natural compounds and support their rational development as therapeutics.

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Quaternary ammonium and phosphonium salts as antimicrobial agents: synthesis, biological evaluation, and mechanistic insights

Nunes Bárbara[1], Cagide Fernando [2], Borges Fernanda [2,3], and Simões Manuel [1]

1. LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal;
2. MedInUP/RISE-Health Department of Biomedicine – Unit of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal;
3. 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

Keywords: *antimicrobial resistance*, *quaternary heteronium salts*, *triphenylphosphonium*, *structure–activity relationship*, and *membrane-active antibacterials*

The rapid emergence of antimicrobial resistance (AMR), particularly among multidrug-resistant (MDR) ESKAPEE pathogens, necessitates antibacterial agents with fundamentally new mechanisms of action. Quaternary heteronium salts (QHSs), encompassing ammonium and phosphonium derivatives, have emerged as versatile amphiphilic scaffolds with significant antibacterial potential. Here, we report a systematic medicinal chemistry study integrating rational design, structure–activity relationship (SAR) analysis, and detailed mechanistic investigation. A focused library of 49 compounds, spanning seven cationic headgroups (triphenylphosphonium (TPP$^+$), methylimidazolium, isoquinolinium, quinolinium, methylpyridinium, pyridinium, and triethylammonium) and alkyl chains (C$_6$–C$_{18}$), was synthesized and evaluated against *Staphylococcus aureus*, including resistant strains. Antibacterial activity was strongly governed by both the cationic core and lipophilicity, with C$_{14}$ derivatives consistently exhibiting optimal potency. TPP$^+$ and methylimidazolium analogues emerged as the most active, albeit with increased cytotoxicity in HepG2 cells [1]. Broad-spectrum screening through CO-ADD identified 33 active compounds, predominantly against Gram-positive bacteria and *Candida albicans*, whereas Gram-negative activity remained limited, consistent with permeability barriers. Toxicological profiling (HEK-293 and hemolysis) revealed a clear chain length–dependent increase in cytotoxicity [2]. Mechanistic studies of the most potent C$_{14}$ derivatives in *S. aureus* demonstrated pronounced, structure-dependent disruption of the bacterial envelope [3]. To improve the therapeutic window, *para*-substituted TPP$^+$ derivatives bearing electron-donating and electron-withdrawing groups (OCH$_3$, F, CF$_3$) were synthesized. Notably, 4-CF$_3$ analogues retained potent antibacterial activity while exhibiting reduced cytotoxicity in NHDF and HepG2 cells. LC-MS/MS confirmed intracellular accumulation, while Seahorse XF and DiOC$_2$(3) assays revealed bioenergetic impairment and membrane depolarization. Collectively, this work establishes optimized TPP$^+$-based QHSs as promising membrane-targeting antibacterial chemotypes with potential against resistant Gram-positive pathogens.

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Targeting Na$_V$ and H$_V$1 channels: discovery of novel small-molecule inhibitors

Martina Piga[1], Nace Zidar[1], Tihomir Tomašič[1]
[1.] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

martina.piga@ffa.uni-lj.si

Keywords: *Na$_V$ and H$_V$1 inhibitors, patch-clamp electrophysiology, structure-based drug design, voltage-gated ion channels*

Voltage-gated ion channels are essential transmembrane proteins that regulate key physiological processes, including neuronal excitability, muscle contraction, and pH homeostasis. Their dysfunction is implicated in numerous diseases, such as chronic pain, epilepsy, cardiovascular disorders, and cancer, making them attractive targets for drug discovery. This work focuses on the design, synthesis, and biological evaluation of novel inhibitors targeting voltage-gated sodium (Na$_V$) and proton (H$_V$1) channels [1].

The first part of the study focused on the Na$_V$1.3 isoform, which is upregulated following nerve injury and associated with neuronal hyperexcitability [2]. Using structure-based drug design, two series of arylsulfonamide derivatives were developed with the aim of improving physicochemical properties while maintaining high potency. Electrophysiological evaluation using automated patch-clamp techniques identified several compounds with nanomolar activity. These inhibitors displayed state-dependent behavior, preferentially targeting the inactivated state of the channel. Importantly, selectivity profiling revealed a favorable safety profile, with several lead compounds showing no activity against the cardiac Na$_V$1.5 isoform.

The second part of the research addressed the discovery of novel H$_V$1 inhibitors through computer-aided drug design. H$_V$1 channels are key regulators of intracellular pH and are frequently overexpressed in highly metastatic cancer cells [3].Virtual screening and molecular docking led to the identification of a new class of inhibitors with a 5-phenyl-2-aminoimidazole scaffold. Subsequent structure–activity relationship (SAR) studies enabled the optimization of analogues with low micromolar IC$_{50}$ values and improved drug-like properties. Notably, biological evaluation demonstrated a correlation between H$_V$1 inhibition and antiproliferative activity in cancer cell lines overexpressing the channel, supporting their potential as anticancer agents. Finally, selectivity profiling and ligand-based pharmacophore modeling were employed to investigate isoform specificity and guide the identification of new Na$_V$1.7 inhibitors for chronic pain treatment. While achieving selectivity remains challenging, the developed models demonstrated potential in virtual screening campaigns, identifying novel candidate molecules for further validation. Overall, this work contributes to the understanding of ion channel modulation and provides novel chemical tools for the study of Na$_V$ and H$_V$1 channels. The results validate the integration of computational design and synthetic chemistry in discovering potent inhibitors, offering promising candidates for the treatment of chronic pain and cancer.

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Using a nanomedicine-based approach as a second chance for tolcapone

Pinto Miguel[1], Fernandes Carlos[1], Machado Cláudia[1], Sarmento Bruno[2], Remião Fernando[3], Otero-Espinar Francisco[4] and Borges Fernanda[5]

1. RISE-Health, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto,
2. Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen 208, 4200-135 Porto
3. Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal;
4. Pharmacology, Pharmacy and Pharmaceutical Technology Department, Faculty of Pharmacy and Institute of Materials (iMATUS), University of Santiago de Compostela (USC), 15782 Santiago de Compostela, Spain;
5. RISE-Health, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal.

miguel.pinto@med.up.pt

Keywords: Parkinson's disease, tolcapone, nanomedicine, polymeric nanoparticles, nanostructured lipid carriers.

Parkinson's Disease (PD) is a movement disorder characterized by neural degeneration and dopamine depletion. The available therapeutic approaches focus on managing the associated motor symptoms by increasing the dopaminergic pool in the brain. Tolcapone, a catechol-O-methyltransferase (COMT) inhibitor, is one of the most effective adjuvant therapies available for managing PD symptoms. However, its use is problematic due to its hepatotoxic profile and pharmacokinetics, requiring close clinical monitoring and multiple daily dosages [1,2]. Nanomedicine has brought several tools capable of improving a drug's therapeutic outcome by exploring the unique characteristics of materials at the nanoscale. In this context, the use of nanoparticles as drug delivery systems enables the modulation of the ADMET properties of drugs, potentially improving their therapeutic efficiency [3]. Thus, the aim of the project was the development of tolcapone-loaded nanoparticles to overcome tolcapone's drawbacks (Figure 1). The first approach was to develop tolcapone-loaded PLGA-based nanoparticles capable of protecting hepatic cells from tolcapone-induced toxicity. After optimization, nanoparticles with sizes around 200 nm were tested in a panoply of hepatotoxicity assays using a human hepatocarcinoma cell line. Nanoparticle formulations proved to decrease tolcapone's cytotoxicity and to counteract ATP depletion and excessive reactive oxygen species production. Plus, tolcapone-loaded PLGA nanoparticles maintained COMT inhibition capabilities. The second approach was the design of lipid-based carriers to improve the oral bioavailability and blood half-life of tolcapone. Tolcapone-loaded nanostructured lipid carriers (NLCs) up to 120 nm were obtained after optimization. *In vitro* assays revealed that NLCs have mucoadhesive properties and are stable in biological mimetic conditions. *In vitro* biological assays demonstrated that NLCs can significantly improve tolcapone permeability across Caco-2 and Caco-2/HT29-MTX cell models, and showed to inhibit COMT. Plus, early *in vivo* toxicological testing using *C. elegans* demonstrated survival percentages > 80% after acute exposure in concentrations up to 100 μM. Together, the project presented two nanomedicine-based solutions with the potential to solve tolcapone's drawbacks, thus giving it a possible second chance for clinical application.

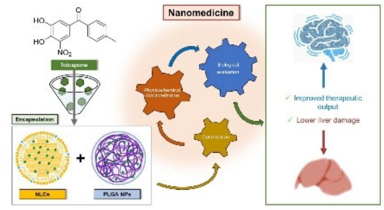

Figure 1. Strategy, development and evaluation of the physicochemical and biological properties of tolcapone-loaded nanoparticles.

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Targeting Autophagy through AMPK: Design, Synthesis, and Biological Evaluation of Novel Allosteric Modulators

Lorenzo Raffellini[1], Roberta Rocca[2], Clementina Manera[1], Sheraz Gul[3], Stefano Alcaro[2], Simona Rapposelli[1]

1. Department of Pharmacy, University of Pisa, Pisa, Italy.
2. Department of Health Science, "Magna Græcia" University of Catanzaro, Catanzaro, Italy.
3. Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Schnackernburgallee 114, 22525 Hamburg, Germany.

lorenzo.raffellini@phd.unipi.it

Keywords: *Autophagy, AMPK, docking, synthesis, ADME-Tox.*

AMP-activated protein kinase (AMPK) is a heterotrimeric serine/threonine kinase that serves as a central regulator of cellular energy homeostasis. Sensitive to fluctuations in intracellular AMP/ATP ratios, AMPK orchestrates a metabolic shift by promoting catabolic processes and inhibiting anabolic ones under energy-deprived conditions [1]. Among the various catabolic pathways regulated by AMPK, autophagy stands out as a pivotal mechanism for maintaining cellular homeostasis. This tightly regulated process of self-digestion ensures cellular survival by recycling damaged organelles and misfolded proteins, particularly under stress conditions such as nutrient deprivation, oxidative stress, or mitochondrial dysfunction [2]. Given its fundamental role in maintaining cellular homeostasis and integrity, impaired autophagy has been linked to the pathogenesis of diverse illnesses, including neuromuscular disorders, metabolic syndrome, and neurodegenerative diseases [3]. As such, pharmacological modulation of AMPK represents a promising strategy to restore autophagic flux and rescue tissue homeostasis to counteract disease progression. In light of the above, we designed a small library of benzimidazole-based derivatives capable of interacting with the allosteric drug and metabolite (ADaM) pocket of AMPK. After *in-silico* evaluation of the proposed structures through a docking study, we synthesized three series of potential ADaM binders. These novel compounds were then tested for their early-ADME-Tox and ecotox properties, with the aim of selecting the most promising ones for progression. Moreover, an *in-vitro* assay was performed to evaluate their potential to activate AMPK (**Figure 1**).

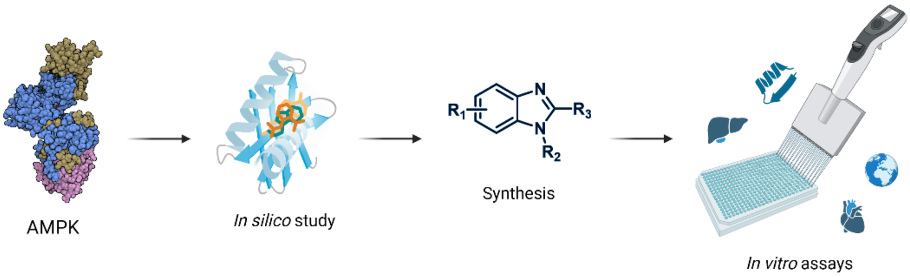

Figure 1. Work-flow schematization.

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Development of Novel Antimalarial Transmission Blocking Agents and Genetic Characterization of Drug Resistance in Mycobacterium abscessus

Benedetta Salvucci[1], Mariangela Biava[1]
1. National Center Rare Diseases, Istituto Superiore di Sanità, Rome, Italy

The discovery and development of novel compounds and innovative therapeutic strategies for infectious diseases remain a formidable challenge due to the rising of drug resistance. This thesis explores this challenge through the development of antimalarial transmission-blocking agents and the characterization of genes contributing to drug resistance in Mycobacterium abscessus.

Malaria remains a global health emergency, with progress in combating the disease stagnating, particularly due to the pandemic COVID-19. The most virulent species, Plasmodium falciparum, has a life cycle involving both mosquitoes (sexual stages) and humans (asexual and sexual stages). The WHO's goal of reducing malaria incidence by 89% by 2030 appears increasingly difficult due to rising resistance of Plasmodium to common antimalarial treatments [1]. Therefore, there is a critical need to develop novel compounds targeting the sexual stages of the parasite's lifecycle to address resistance mechanisms in the asexual stages, which are the primary targets of conventional antimalarial drugs [2]. Transmission-blocking compounds that inhibit parasite development in both human hosts and mosquito vectors offer a promising approach [3]. Pursuing this aim, we synthesized analogues of MMV1580843, identified in a high-throughput screening as a potent and selective gametocytocidal compound [4,5]. The goal of this PhD thesis is to design and synthesize new pyrrole- and imidazole-based derivatives by modifying the substitutions around the central core, to enhance transmission-blocking activity.

Infections caused by non-tuberculous mycobacteria, particularly Mycobacterium abscessus (Mab), continue to present significant therapeutic challenges due to intrinsic resistance to most antibiotics. Mab is an opportunistic pathogen responsible for severe pulmonary infections, especially in immunocompromised individuals. This study aimed to identify potential therapeutic targets to enhance the efficacy of Bedaquiline and SQ109, by performing transposon mutagenesis and sequencing screen (TnSeq). The focus was on discovering conditionally essential genes that may play a critical role under host-like conditions, compared to those identified in standard culture media. To simulate a host-like environment, we developed an in vitro infection model using air-liquid interface (ALI) culture. This approach aims to uncover potential targets for improving treatment strategies against Mab and addressing the growing threat of this challenging pathogen [6,7].

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LIST OF POSTER COMMUNICATIONS (PCs)

Number	Author	Title
PC1 MORE	Francesca Arrighi	*From Small Molecules to Big Outcomes: Advancing Benzo[b]thiophenes in Neurodegeneration*
PC2 MORE	Giulia Atzeni	*Identification of dual NS3 protease-helicase inhibitors as broad-spectrum antivirals against WNV and ZIKV*
PC3 MORE	Gyde Bandowski	*Synthesis of γ-monoalkylated Triphosphate Analogues to inhibit SARS-CoV-2 Replication-Transcription Complex*
PC4 MORE	Mihail Lucian Birsa	*Synthetic flavonoid – an alternative solution to combat eskape pathogens*
PC5 MORE	Roberta Bivacqua	*Chemical Exploration of N-(2-phenyl-1,3-thiazol-5-yl)benzamides as Inhibitors of Influenza A/H1N1 Hemagglutinin-Mediated Viral Fusion*
PC6 MORE	Fernando Cagide-Fagín	*Hydroxypyridin-4-one-based ligands as multifunctional therapeutics for neurodegenerative diseases*
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PC15 MORE	Jan Konecny	*Optimization of pyrrolo[3,2-d]pyrimidine derivatives as antitubercular agents through drug-driven design*
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PC26 MORE	Alessia Onali	*Novel Thiazolidinone-Isatin Hybrids as Potent hCA IX Inhibitors Enhance Doxorubicin-Induced Apoptosis in Hypoxic Glioblastoma Cells*

PC27 MORE	Maria Rosaria Paravati	*From Viral Proteins to Host Targets: Unveiling Casein Kinase 2 as the Antiviral Mechanism of the [4,7]-Phenanthroline Scaffold*
PC28 MORE	Ludwika Piwowarczyk	*Nanocarrier-based delivery of 20-hydroxyecdysone modulates IL-17/IL-23 signaling in psoriasis model*
PC29 MORE	Lara Prilop	*Combining DHODH inhibitors and pronucleotides to block viral replication*
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From Small Molecules to Big Outcomes: Advancing Benzo[*b*]thiophenes in Neurodegeneration

Francesca Arrighi [a,1], Emanuela Berrino [a,b,1], Paolo Guglielmi [a,*], Simone Carradori [c,*], Guya Diletta Marconi [d], Jacopo Pizzicannella [d], Simone Guarnieri [e], Tiziano Tuccinardi [f], Giulio Poli [f], Federico Pepi [a], Anna Troiani [a], Chiara Salvitti [a], Alessia Di Noi [a], Michele Coluccia [a], Giorgio Buttitta [a], Virginia Pontecorvi [a,g], Arianna Granese [a], Paola Chimenti [a], Daniela Secci [a], Anel Petzer [h,i], Jacobus Petzer [h,i], Francesca Diomede [d]

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

[b] *Department of Life Science, Health, and Health Professions, Link Campus University, Via del Casale di San Pio V, 44 – 00165, Rome, Italy*

[c] *Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy*

[d] *Department of Innovative Technologies in Medicine & Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy*

[e] *Department of Neuroscience, Imaging and Clinical Sciences, "G. d'Annunzio" University of Chieti-Pescara, Via Luigi Polacchi 11, 66100 Chieti, Italy*

[f] *Department of Pharmacy, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy*

[g] *Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Corso della Repubblica 79, 04100, Latina, Italy*

[h] *Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom, 2520, South Africa*

[i] *Pharmaceutical Chemistry, School of Pharmacy and Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa*

francesca.arrighi@uniroma1.it

Keywords: Parkinson's disease, hMAO-B inhibitors, 2-aroylbenzothiophenes, Oxidative stress, Neuroprotection

Neurodegenerative disorders are an increasing challenge in ageing societies. In Parkinson's disease, the overactivity of hMAO-B promotes oxidative stress and dopaminergic neurodegeneration, making selective hMAO-B inhibition a promising therapeutic strategy. Building on previous studies on benzofuran and benzothiophene-3-ol scaffolds, this work investigates 2-aroylbenzothiophene derivatives as new candidates for neurodegeneration-oriented drug discovery[1].

Compounds 4, 11, and 12 emerged as the most promising hMAO-B inhibitors. In preliminary cellular assays, compound 4 showed cytotoxicity and increased ROS production, whereas compounds 11 and 12 were well tolerated up to 100 µM, with a safety profile comparable to Selegiline. Notably, compound 12 reduced basal ROS levels and showed modest neuroprotective effects in SH-SY5Y cells exposed to 6-OHDA. Molecular modelling studies supported the proposed binding modes[2].

Overall, this study identifies a new class of selective nanomolar hMAO-B inhibitors, whose activity is strongly influenced by aryl substitution.

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Identification of dual NS3 protease-helicase inhibitors as broad-spectrum antivirals against WNV and ZIKV

Atzeni Giulia[1,2], Lupia Antonio [1], Emmolo Roberta [1], Corona Angela [1], Nieddu Salvatore [1], Esposito Francesca Sanna Erica [1], Onali Alessia [1], Demuru Laura [1], Meleddu Rita [1], Cottiglia Filippo [1], Tramontano Enzo [1] and Maccioni Elias [1]

1. Department of Life and Environmental Sciences, University of Cagliari, 09042 Monserrato, Italy
2. National PhD Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, University of Pavia, 27100, Italy

giulia.atzeni@unica.it

Keywords: Flavivirus, helicase, protease, West Nile, ZIKV

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

The 11-kb positive-sense, single-stranded RNA (ssRNA) genome is translated into a single polyprotein, which is subsequently cleaved into three structural proteins (C, prM/M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Among them, NS3 is a multifunctional, highly conserved flavivirus protein that integrates protease and helicase functions and is indispensable for viral maturation, replication, and infectivity [2]. While most antiviral efforts focus on single-target inhibitors, dual inhibitors targeting both protease and helicase activities offer the potential for enhanced efficacy and reduced resistance. Using Computer-Aided Drug Design (CADD), we identified a series of compounds capable of inhibiting both NS3 helicase and protease activities of West Nile virus (WNV) and Zika virus (ZIKV) at low micromolar concentrations. Scaffold optimization through multiple structural modifications is ongoing to improve antiviral potency and reduce cytotoxicity. Pure enantiomers of the most active compound were isolated and evaluated, showing no significant difference in biological activity. These findings underscore the promise of broad-spectrum dual NS3 inhibitors as an effective antiviral strategy against flaviviruses.

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Synthesis of γ-monoalkylated Triphosphate Analogues to inhibit SARS-CoV-2 Replication-Transcription Complex

Gyde Bandowski[1], Paolo Malune[2], Francesca Esposito[2], Antonio Lupia[2], Simona Distinto[2], Elias Maccioni[2], Xiao Jia[1,3], Giuliano Kullik[1], Enzo Tramontano[2], Chris Meier[1,4]

1. Department of Chemistry, Organic Chemistry, Faculty of Sciences, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany;
2. Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, Cagliari, 09124, Italy;
3. State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;
4. Centre for Structural Systems Biology (CSSB), Hamburg, DESY Campus, Notkestrasse 85, 22607 Hamburg, Germany

gyde.bandowski@uni-hamburg.de

Keywords: *antiviral, SARS-CoV-2, nucleotides, RdRp complex*

Acyclic nucleoside phosphonates, such as (*S*)-HPMPC, have shown significant antiviral activity against a range of viruses [1]. Based on the structure of (*S*)-HPMPC, we synthesised a series of γ-monoalkylated nucleoside phosphonate diphosphates and their prodrugs with different nucleobases and alkyl chain lengths, by applying the *H*-phosphonate route for the synthesis of Tri*PPP*ro-compounds. This way, the enzymatic phosphorylation to the biological active triphosphate analogue is bypassed [2]. The γ-monoalkylated compounds were found to inhibit the viral RNA-dependent RNA polymerase (RdRp) complex of SARS-CoV-2 with a novel unusual mechanism of action. Enzymatic PAGE-based assays of the minimal replication-transcription complex (RTC) of SARS-CoV-2 showed complete loss of enzymatic function of the RdRp with no incorporation of the nucleotide analogues and no competition with natural nucleoside triphosphates. IC_{50} values in the low micromolar range were determined in dependence on the length of the γ-alkyl group. Using multiple techniques, we were able to identify the mechanism of action in the compound-induced dissociation of the nsp7 cofactor from the nsp12 subunit. Molecular docking confirmed the binding site, identifying putative amino acid residues relevant for compound interaction. To further investigate of the novel mechanism of action, other nucleotide analogues were synthesised, to determine which structural motifs of the compounds are involved in binding and thus are responsible for their inhibitory action. IC_{50} values for γ-monoalkylated nucleotide analogues with different nucleoside cores and varying numbers of phosphate moieties were evaluated.

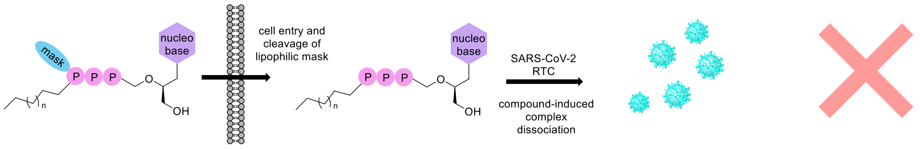

Figure 1. γ-Monoalkylated nucleoside triphosphate analogues induce dissociation of the SARS-CoV-2 replication-transcription complex *in vivo*. Cell entry is possible through the use of prodrugs.

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Synthetic Flavonoid - An Alternative Solution to Combat ESKAPE Pathogens

Mihail Lucian Birsa[1]

1. Department of Chemistry, “Alexandru Ioan Cuza” University of Iasi, Romania

lbirsa@uaic.ro

Keywords: *ESKAPE pathogens, synthetic flavonoids, anti-biofilm activity, synergistic effect*

ESKAPE pathogens are considered as global threats to human health. The discovery of new molecules for which these pathogens have not yet developed resistance is a high medical priority [1]. Synthetic flavonoids are good candidates for developing new antimicrobials [2, 3]. Therefore, we report here the potent in vitro antibacterial activity of BrCl-flav (Figure 1), a representative of a new class of synthetic tricyclic flavonoids. Minimum inhibitory/bactericidal concentration, time kill and biofilm formation assays were employed to evaluate the antibacterial potential of BrCl-flav. The mechanism of action was investigated using fluorescence and scanning electron microscopy. A checkerboard assay was used to study the effect of the tested compound in combination with antibiotics. Our results showed that BrCl-flav displayed important inhibitory activity against all tested clinical isolates, with MICs ranging between 0.24 and 125 µg/mL. A total kill effect was recorded after only 1 h of exposing *Enterococcus faecium* cells to BrCl-flav. Additionally, BrCl-flav displayed important biofilm disruption potential against *Acinetobacter baumannii*. Those effects were induced by membrane integrity damage. BrCl-flav expressed synergistic activity in combination with penicillin against a MRSA strain. Based on the potent antibacterial activity, low cytotoxicity and pro-inflammatory effect, BrCl-flav has good potential for developing new effective drugs against ESKAPE pathogens [4].

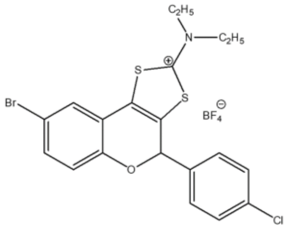

Figure 1. The chemical structure of BrCl-flav

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Chemical Exploration of *N*-(2-phenyl-1,3-thiazol-5-yl)benzamides as Inhibitors of Influenza A/H1N1 Hemagglutinin-Mediated Viral Fusion

Raso Miriam[1], **Bivacqua Roberta**[1,2], Romeo Isabella[2], Barreca Marilia[1], Raimondi Maria Valeria[1], Spanò Virginia[1], Alcaro Stefano[2], Barraja Paola[1], Cagno Valeria[3] and Montalbano Alessandra[1]

1. Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, 90123, Palermo, Italy.
2. Dipartimento di Scienze della Salute, Università "Magna Græcia", 88100, Catanzaro, Italy.
3. Institute of Microbiology, University Hospital of Lausanne, University of Lausanne, 1011, Lausanne, Switzerland.

roberta.bivacqua@studenti.unicz.it

Keywords: *Influenza A virus, Hemagglutinin, A/H1N1 Replication, Viral Entry Inhibitors, Structure-Activity Relationship.*

The rapid evolution of influenza A virus (IAV) and the emergence of drug-resistant strains with reduced susceptibility to currently available antivirals emphasize the need for innovative therapeutic approaches [1]. The viral hemagglutinin (HA) glycoprotein plays a crucial role in the early stages of the viral life cycle by mediating host cell attachment and membrane fusion, making it an attractive target for the development of novel entry inhibitors [2].

In our previous studies, we identified a series of *N*-(2-phenyl-1,3-thiazol-5-yl)benzamide derivatives (**1**) displaying promising antiviral activity against influenza viruses, with potent inhibition of A/H1N1 replication in MDCK cells and EC_{50} values in the submicromolar range (unpublished results). The A/H1N1 pseudovirus entry assay further supported a mechanism of action consistent with inhibition of the HA-mediated viral entry process.

Based on these encouraging results, we started an exploration of the chemical space around the 1,3-thiazole scaffold through the design and synthesis of novel chemotypes (**2**), focusing on the key positions of chemical variability within the molecule (Figure 1). This chemical diversification is expected to provide further insight into the structural features related to the antiviral activity and the structure–activity relationships within this promising class of HA-targeting inhibitors.

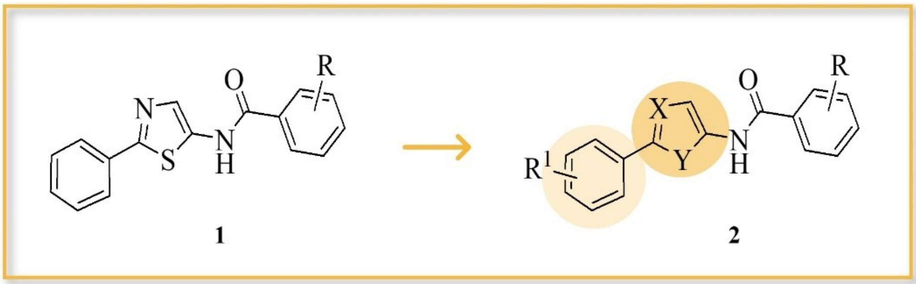

Figure 1. Structural modifications of *N*-(2-phenyl-1,3-thiazol-5-yl)benzamides

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Hydroxypyridin-4-one-based ligands as multifunctional therapeutics for neurodegenerative diseases

Fernando Cagide[1], Carla Lima[2], Sofia Benfeito[1], Daniel Chavarria[1], Lisa Sequeira[1], Bárbara Albuquerque[1], Paula Serrão1, Patrício Soares-da-Silva1, Fernanda Borges[1,2]

1. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
2. RISE-Health, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

fernando.fagin@med.up.pt

Keywords: *Catechol-O-methyltransferase, hydroxypyridin-4-one scaffold, Iron chelation; Multitarget-directed ligands, Parkinson's disease*

Neurodegenerative disorders are complex, multifactorial diseases driven by interconnected pathological processes, including neurotransmitter imbalance, oxidative stress, mitochondrial dysfunction, and metal dyshomeostasis. In Parkinson's disease (PD), dopamine depletion and iron accumulation are two closely linked hallmarks that contribute to disease progression. Catechol-O-methyltransferase (COMT) plays a central role in dopamine degradation, and its inhibition is a validated therapeutic strategy for PD. However, current COMT inhibitors such as tolcapone, entacapone, and opicapone are limited by hepatotoxicity and poor brain penetration. Their activity depends on a nitrocatechol motif that coordinates the enzyme's magnesium cofactor, but the nitro group is also associated with toxicity, underscoring the need for safer alternatives.

Here, we explore multitarget-directed ligands (MTDLs) as a strategy to address these converging pathological mechanisms. MTDLs are designed to combine multiple pharmacophores within a single chemical entity to simultaneously modulate dopamine dysregulation and iron-induced oxidative stress. Building on our previously identified brain-penetrant and selective COMT inhibitor (compound 9), we designed and synthesized a small library of hydroxypyridin-4-one-based derivatives incorporating iron-chelating motifs. The compounds were evaluated for COMT inhibition, metal-binding selectivity, and cytotoxicity. Several derivatives showed selective COMT inhibition, preferential binding of Cu(II) over Fe(II) and Mg(II), and no cytotoxicity at 25 μM. The results obtained so far will be presented in this communication.

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Bioisosteric replacement of the maleimide warhead in a selective covalent NOX4 inhibitor

Inês Lima[1], Mariana Castelôa[1], Sofia Benfeito[1], Fernanda Borges[1,2], Daniel Chavarria[1]

1. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
2. RISE-Health, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

up201904940@fc.up.pt

Keywords: *NADPH oxidase*; *Targeted covalent inhibitors; Cysteine targeting; Neurodegenerative diseases.*

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) is one of the seven members of the NOX family and catalyzes the formation of reactive oxygen species. NOX4 has been implicated in neurodegenerative diseases (NDs), being a key player in pathological events such as ferroptosis, neuroinflammation, and protein aggregation [1]. Several small molecules have been identified as NOX inhibitors, but they usually present poor homolog selectivity, assay-interfering effects, unfavourable drug-like properties, and/or cytotoxicity. Therefore, the development of new selective, non-interfering inhibitors of NOX4 is of upmost importance to clearly understand its biological role and to validate it as a drug target for NDs [2, 3]. Recently, the team developed MAC18, a covalent, selective NOX4 inhibitor with µM potency, targeting a cysteine residue within the enzyme's dehydrogenase domain. Structurally, MAC18 is composed of a maleimide warhead and a heterocyclic ring connected through a linker.
As part of our drug discovery program, this work aims at developing MAC18 derivatives bearing maleimide bioisosteres with moderate electrophilicity for NOX inhibition screenings. Following the optimization of the synthetic strategy, a small library of compounds was successfully synthesized. The results obtained so far will be presented in this communication.

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Novel Benzofuran-Donepezil derivatives to dually inhibit monoamine oxidases and cholinesterases: a multifunctional strategy against neurodegenerative diseases

Coluccia Michele[1], Arrighi Francesca[1], Vernile Sofia[1], Giorgio Buttitta[1], Paolo Guglielmi[1], Arianna Granese[1], Paola Chimenti[1] and Daniela Secci[1]

1. Department of Drug Chemistry and Technologies, Sapienza University of Rome, Rome, Italy;

michele.coluccia@uniroma1.it

Keywords: *neurodegeneration, hMAO, acetylcholinesterase, butyrylcholinesterase*

Neurodegenerative diseases (with Alzheimer's and Parkinson's diseases as the most prominent representatives) are among the heaviest burdens on healthcare systems all over the world and possess complex and heterogeneous pathogeneses, far from a complete understanding [1]. Among the various factors involved, human monoamine oxidases (hMAOs) and cholinesterases (ChEs: acetylcholinesterase, AchE, and butyrylcholinesterase, BChE) occupy a remarkable position. In fact, both classes of enzymes are clinically targeted in the treatment of neurodegenerative diseases, and the development of dual inhibitors has recently emerged as an interesting approach to enhance the therapeutic efficacy of novel drug candidates [2]. For these reasons, we have decided to develop a series of novel compounds to dually inhibit these enzymes. The design of the scaffold (see **Figure 1**) took inspiration from the benzofuran nucleus, which we have successfully employed for the design of potent and selective 2-aroylbenzofuran based hMAO-B inhibitors [3]. The benzofuran portion notably resembles the indanone core of the AChE inhibitor donepezil, currently used for the symptomatic treatment of AD. This nucleus was then fused with the piperidine moiety of donepezil through a N-ethyl carboxyamidic linker. The compounds were obtained via a three-step synthetic process and, after silica gel column chromatography, were chemically characterised using 1H and 13C NMR. The inhibitory activity of the derivatives was then evaluated in vitro through spectrophotometric enzymatic inhibition assays against hMAOs and ChEs.

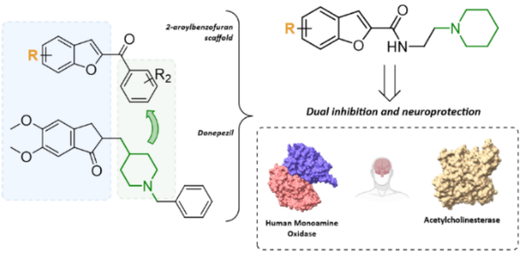

Figure 1. Design of the benzofuran-donepezil derivatives

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Targeting CtBP mediated oncogenic transcriptional activity with structure guided ligand

El Hadri Chaimae[1,2,4], DeBacker Robin[3,4], Savvas Savvides[3,4], Steven Goossens[1,4], Serge Van Calenbergh [2,4]

1. Unit for Translational Research in Oncology (UTRiO), Ghent University, Ghent, Belgium
2. Laboratory for Medicinal Chemistry, Ghent University, Ghent, Belgium
3. Unit for Structural Biology, VIB Ghent University, Ghent, Belgium
4. Cancer Research Institute Ghent (CRIG), Ghent, Belgium

chaimae.elhadri@ugent.be

Keywords: CtBP, HIPP, transcriptional factor

C-terminal Binding Proteins (CtBP1/2) are NADH-dependent transcriptional corepressors frequently overexpressed in cancer, where they drive epithelial mesenchymal transition (EMT) and repress tumor suppressor programs (CDH1, BRCA1)[1-2]. However, the interplay between their catalytic activity, oligomerization, and transcriptional function remains poorly understood. Here, we report the structure guided development of new CtBP inhibitors derived from 2-hydroxyimino 3-phenylpropanoic acid (HIPP)[3]. The most potent compounds display nanomolar inhibition of CtBP dehydrogenase activity ($IC_{50} \approx 20$ nM). Biophysical and structural studies (thermal shift, ITC, and X-ray crystallography) reveal high affinity binding and show that the enzymatic inhibition is coupled to perturbation of CtBP oligomerization, a key determinant of its transcriptional activity. In breast and ovarian cancer models, the ethyl esters of these compounds reduce viability ($IC_{50} \approx 15$ μM) and induce transcriptional reprogramming consistent with CtBP modulation. These results provide insight into the relationship between CtBP catalytic activity and transcriptional regulation, and support further exploration of CtBP as a therapeutic target.

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Integrated Experimental and Computational Analysis of Benzimidazole α-Glucosidase Inhibitors for Pathway-Informed Design

Ferro Elsi [1], Laus Antonio [1], Atzeni Rossano [1], Pieroni Enrico [1], Valentini Maria [1], Massimo Pisu [1]

1. CRS4 (Center for Advanced Studies, Research and Development in Sardinia), Pula, Italy

elsi@crs4.it

Keywords: α-glucosidase inhibition, benzimidazole derivatives, molecular modeling, structure–activity relationship

Inhibition of α-glucosidase represents an established therapeutic strategy for the control of postprandial hyperglycemia in type 2 diabetes. Currently available inhibitors are often associated with adverse effects, therefore the identification of novel compounds with improved efficacy and safety profiles remains an important challenge.

This work focuses on the combined experimental and computational investigation of benzimidazole derivatives as potential α-glucosidase inhibitors, with the aim of enabling pathway-informed optimization and rational design strategies. Selected compounds, synthesized through an efficient and environmentally friendly protocol, have shown promising inhibitory activity against α-glucosidase, with limited effects on α-amylase and no significant cytotoxicity in cellular models [1].

On the computational side, ongoing efforts are aimed at elucidating the molecular determinants of ligand binding and supporting the interpretation of experimental observations. In our approach, structure-based molecular modeling approaches are employed, including molecular docking to predict binding modes, classical molecular dynamics simulations to probe the stability of protein–ligand interactions, and cutting-edge enhanced sampling techniques [2] to explore conformational variability and particularly binding free/energy landscapes.

Overall, this work aims to provide a framework for the rational characterization and prioritization of candidate α-glucosidase inhibitors, while contributing to pathway-informed optimization and design strategies.

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In Silico Screening of Natural Product Libraries for α-Glucosidase Inhibitors Targeting Saccharomyces cerevisiae

Greco Ilario[1,2], Rocca Roberta[1,2] and Alcaro Stefano[1,2]

1. Dipartimento di Scienze della Salute, Università “Magna Græcia” di Catanzaro, Campus Universitario “S. Venuta”, Viale Europa, 88100 Catanzaro, Italy;
2. Associazione CRISEA – Centro di Ricerca e Servizi Avanzati per l’Innovazione Rurale, Località Condoleo, 88055 Belcastro (CZ), Italy.

ilario.greco@studenti.unicz.it

Keywords: *α-glucosidase, diabetes, virtual screening, molecular docking, binding affinity*

α-Glucosidase is a key enzyme involved in carbohydrate metabolism and represents an important therapeutic target for the management of type 2 diabetes [1], as it catalyzes the final step in the breakdown of complex carbohydrates into glucose. In this study, a virtual screening approach [2] was applied to four natural compound libraries to identify potential inhibitors of α-glucosidase from *Saccharomyces cerevisiae*. Molecular docking simulations were performed using the Glide software [3] (Schrödinger suite) to evaluate the binding affinity and interaction patterns of the selected compounds within the enzyme active site. Ligands were ranked according to their Glide docking scores, and their binding modes were analyzed to assess interactions with key catalytic residues and overall binding stability. The screening identified several natural compounds with promising inhibitory potential, characterized by favorable docking scores and stable interactions within the active site. These results highlight natural product libraries as a valuable source of bioactive scaffolds and support the use of Glide-based virtual screening approaches for the identification of novel α-glucosidase inhibitors.

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Phosphorite: useful industrial waste for one-pot green synthesis of quinolones

Alessandro Gualtieri[1,2], Rosa Scarpelli[1,2], Monica Nardi[1,2], Giorgia Sinopoli[1,3] , Salvatore Procopio[3], Antonio Procopio[1,2]

1. Departement of Health Sciences, University Magna Graecia of Catanzaro, Campus S. Venuta, Viale Europa, loc. Germaneto, 88100 Catanzaro (Italy)
2. "AGreenFood" Research Center, University "Magna Græcia" of Catanzaro, Campus Universitario "S Venuta", loc. Germaneto, 88100 Catanzaro (Italy)
3. ARPA-CALABRIA (Regional Agency for Environmental Protection of Calabria), loc. Giovino, 88100 Catanzaro, Italy

alessandro.gualtieri@unicz.it

Keywords: *phosphorite, quinolones, green chemistry.*

Since 1928, industrial activities of Crotone have been characterized by the presence of the most important Italian chemical industries. The first production was of sulfuric acid, used for extraction and processing of phosphorite rocks rich of ^{238}U and ^{232}Th. [1] The trial was the basis to produce phosphoric acid, useful for fertilizers, detergents, feed, food additives and pesticides. [2,3] The industrial waste could create a problem from an environmental impact point of view if it is not possible to enhance its structural chemical-physical characteristics. The conversion of waste materials into valuable products or green chemicals using green chemistry principles is an important goal to promote an increasingly eco-sustainable society.

In the present study, the performance of industrial wastes as the fumace slag was investigated for the synthesis one-pot of a heterocyclic classes of organic compounds biologically active as 4-quinolones (Figure 1) [4].

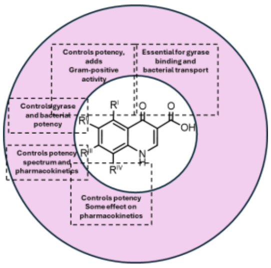

Figure 1. "Structure-activity" relationship of 4-quinolones

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Synthesis, molecular modeling and assessment of anticancer activity of new CYP17A1 inhibitors of the D2AAK1M series

Michał K. Jastrzębski[1], Tomasz M. Wróbel[1], Agnieszka Korga-Plewko[2], Magdalena Iwan[3], Joanna Kubik[2] and Agnieszka A. Kaczor[1]

1. Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Faculty of Pharmacy, Medical University of Lublin, 4A Chodźki St., PL-20093 Lublin, Poland
2. Independent Medical Biology Unit, Medical University of Lublin, 8b Jaczewski Street, 20-090 Lublin, Poland
3. Department of Toxicology, Faculty of Pharmacy, Medical University of Lublin, Chodźki 8, 20-093 Lublin, Poland.

michal.jastrz1998@gmail.com

Keywords: *CYP17A1, prostate cancer, molecular docking*

Prostate cancer (PCa) is the second leading cause of cancer-related mortality in men. A key therapeutic target in castration-resistant prostate cancer (CRPC) is the CYP17A1 enzyme, which catalyzes androgen biosynthesis. Abiraterone, currently used in clinical practice, possesses a steroidal scaffold, which limits its selectivity and leads to adverse effects. This work presents the development of a new series of non-steroidal inhibitors, D2AAK1M (Fig. 1), representing an evolution of pyridine-indole hybrids [1,2].

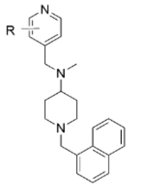

Figure 1. Structure of D2AAK1M derivatives.

The designed compounds were based on a 4-substituted pyridine scaffold (the heme-binding group) connected *via* an aliphatic linker to a naphthalene moiety. PASS analysis demonstrated high probability of both antitumor activity and selective inhibition of steroidogenic enzymes. Molecular docking studies (SeeSAR) confirmed that D2AAK1M derivatives occupy the same binding pocket as abiraterone. The molecules adopt an optimal pose: the scaffold is oriented at a 60° angle relative to the heme, with the pyridine nitrogen atom coordinating the iron at an approximately perpendicular angle. *In vitro* experiments using PCa cell lines (LNCaP, DU-145, PC3) and BJ fibroblasts demonstrated that certain compounds selectively influenced cell viability. These agents significantly reduced the viability of the LNCaP (AR+) cell line at 100 µM. Simultaneously, they remained inactive against AR-negative lines (Du-145, PC3) and exhibited no toxicity toward normal cells. Selective effects in AR+ support the hypothesis of a mechanism based on the inhibition of androgen production. The D2AAK1M series represents a promising starting point for the development of novel anticancer drugs with an improved safety profile.

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A novel cariprazine-derived D2/5-HT3 receptor modulators with potential implications for the treatment of schizophrenia and depressive symptoms

Radomir Juza[1], Tomas Petrasek[1], Ondrej Soukup[2], and Jan Korabecny[2]
1. National Institute of Mental Health, Topolova 748, 250 67 Klecany, Czech Republic;
2. Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic;

radomir.juza@nudz.cz

Keywords: *schizophrenia, depression, D2 receptor, 5-HT3 receptor, cariprazine*

Schizophrenia is a serious mental disorder with approximately 1% lifetime prevalence [1]. Currently used antipsychotic drugs are encumbered with many limitations. In addition to frequently occurring side effects [2], in most cases they alleviate predominantly positive symptoms. This effect can be explained by the fact that all registered neuroleptics were primarily discovered to target D$_2$ receptors (D$_2$R) [3]. It has been shown that 5-HT$_3$ receptor (5-HT$_3$R) antagonists, known as setrons, used as add-on treatment to standard antipsychotics, improve clinical symptoms of schizophrenia, especially negative and cognitive symptoms. Furthermore, setrons decrease extrapyramidal side effects of antipsychotics (e.g., tardive dyskinesia) [4]. Another issue observed in schizophrenic patients is the high prevalence of comorbid diseases such as depression. The lifetime prevalence of depression in schizophrenia is reported to be around 40% [5]. Many clinical studies suggest the use of setrons to suppress depressive symptoms [6]. Based on these observations, we present a novel family of D$_2$/5-HT$_3$ receptor ligands combining various arylpiperazine scaffolds and aryl(piperazine-1-yl)methanone moieties tethered by an aliphatic linker, aiming at a comprehensive therapeutic effect in the treatment of schizophrenia and depressive symptoms. This approach merges affinity towards both D$_2$ and 5-HT$_3$ receptors within a single molecular framework. In this contribution, we report the design and synthesis of the compounds in detail, together with their in vitro evaluation, including cytotoxicity assessment, prediction of blood–brain barrier penetration, and determination of affinities and functional activities at the desired receptors.

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Optimization of Pyrrolo[3,2-*D*]Pyrimidine Derivatives as Antitubercular Agents through Drug-Driven Design

Konecny Jan [1], Finger Vladimir [1,2], Novak Martin [1], Roh Jaroslav [2] and Korabecny Jan [1]

1. Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
2. Faculty of Pharmacy in Hradec Kralove, Charles University, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic

konecny77@seznam.cz

Keywords: *pyrimidine, Mycobacterium tuberculosis, DprE1*

Tuberculosis (TB) remains one of the ten leading causes of death worldwide and is caused by the infectious pathogen *Mycobacterium tuberculosis* (*Mtb*). According to the World Health Organization (WHO), approximately 10.7 million new TB cases and 1.23 million deaths were reported in 2025.[1] One of the major obstacles in TB therapy is the growing prevalence of *Mtb* strains resistant to both first-line drugs (isoniazid, pyrazinamide, ethambutol, and rifampicin) and second-line agents (fluoroquinolones, amikacin, bedaquiline, pretomanid, delamanid, etc.). This alarming trend underscores the urgent need to develop new anti-TB drugs with novel mechanisms of action to improve treatment outcomes and overcome drug resistance. In our previous work, we identified a purine-based compound, K2032, with strong anti-TB activity, demonstrating a minimum inhibitory concentration (MIC$_{99}$) of 1 µM against the H$_{37}$Rv strain (for comparison, isoniazid exhibits an MIC$_{99}$ of 0.5 µM). Whole-genome sequencing of resistant strains revealed that K2032 functions as a noncovalent inhibitor of DprE1. In a subsequent study, we employed a scaffold-hopping strategy to simplify the purine core to a 5*H*-pyrrolo[3,2-*d*]pyrimidine scaffold. Within this compound series, we discovered the highly potent derivative K2653, which showed high activity against both drug-susceptible *Mtb* strains (MIC$_{99}$ = 2 µM) and extensively drug-resistant *Mtb* strains (MIC$_{99}$ = 2 µM). However, K2653 displayed approximately 20-fold higher cytotoxicity (IC$_{50}$ in HepG2 cells = 3.5 µM) compared with the most potent purine derivative, K2032 (IC$_{50}$ in HepG2 cells = 63.8 µM). *In vivo* pharmacokinetic studies conducted in rats enabled the identification of plasma metabolites responsible for the increased toxicity of K2653. Through targeted structural modifications, including the incorporation of deuterium into the K2653 scaffold, we successfully improved its cytotoxicity profile.[2]

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20-Hydroxyecdysone modulates inflammatory cytokines in human psoriasis keratinocytes, PHEK cells

Krajka-Kuźniak Violetta [1], Kleszcz Robert [1], Majchrzak-Celińska Aleksandra [1], Musielak Ewelina [1], Gajewska Julia [1], and Piwowarczyk Ludwika [2]

[1] Poznan University of Medical Sciences, Department of Pharmaceutical Biochemistry, Rokietnicka 3, Poznań, Poland
[2] Poznan University of Medical Sciences, Department of Pharmaceutical Chemistry, Rokietnicka 3, Poznań, Poland

vkrajka@ump.edu.pl

Keywords: psoriasis, 20-hydroxyecdysone, keratinocytes, interleukins

Psoriasis is a chronic, immune-mediated skin disorder marked by epidermal hyperproliferation, abnormal differentiation, and persistent inflammation driven by dysregulated cytokine signaling [1]. Keratinocytes act not only as targets of immune activation but also as active contributors, releasing pro-inflammatory mediators that sustain local inflammation. 20-Hydroxyecdysone (20-HE), a natural phytoecdysteroid, has attracted interest for its anti-inflammatory, antioxidant, and cytoprotective effects, suggesting potential relevance in psoriasis.

In the preliminary study, human epidermal keratinocytes (HEKs) and keratinocytes derived from psoriasis patient (PHEKs) were exposed to 20-HE under standard culture conditions for 48 hours. Following treatment, cell culture supernatants were analyzed using the MAGPIX® multiplex bead-based immunoassay. The cytokine panel included GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-12p70, IL-13, IL-17A, IL-18, IL-22, IL-23, and TNF-α, covering key mediators involved in inflammatory signaling networks relevant to psoriasis pathogenesis [2]. Treatment with 20-HE modulated several pro-inflammatory cytokines, including GM-CSF, IL-2, IL-4, IL-5, IL-17A, IL-22, and TNF-α, with more pronounced effects in psoriatic keratinocytes. These changes reflect the regulation of pathways associated with keratinocyte activation, immune cell crosstalk, and epidermal inflammation.

In conclusion, 20-HE demonstrates notable anti-inflammatory and immunomodulatory activity in psoriatic keratinocytes. These findings support its potential as a candidate for further investigation in the development of novel therapeutic approaches targeting keratinocyte-mediated inflammation in psoriasis.

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3,5-Diiodosalicylaldehyde: A Valuable Building Block for Novel Antifungal Agents

Krátký Martin[1], Vávrová Pavlína[1], and Konečná Klára[1]

1. Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

martin.kratky@faf.cuni.cz

Keywords: *3,5-diiodosalicylaldehyde, antifungal activity, hydrazones, imines,* and *synthesis*

The increasing global burden of fungal infections, combined with emerging resistance and toxicity of used drugs, highlights the urgent need for new antifungal agents. Current pipeline remains insufficient to adequately address priority fungal pathogens. Therefore, the synthesis of new molecules is essential for identification of novel chemotypes for safer and more effective antifungals [1]. Iodinated aromatics [2] and salicylic scaffolds [3] represent attractive motifs in antifungal design, demonstrating potency against various species, including resistant isolates. That is why we investigated iodinated salicylic-based compounds as promising antifungal agents. We identified a condensate of 3,5-diiodosalicylaldehyde with D-cycloserine with potent activity, with MIC values ≥3.9 µM for yeasts and ≥15.62 µM for moulds, accompanied by mild inhibition of Gram-positive bacteria and mycobacteria (MIC ≥62.5 µM). This finding inspired an extensive structure–activity relationship study, focused on the positional isomer of 3,5-diiodosalicyl-aldehyde, modification of the phenolic group, the linker between the aldehyde and the second part of the molecule (imine, hydrazone, hydrazide–hydrazone), and substituent R (Figure 1). The results highlight the importance of the 3,5-diiodosalicylidene moiety. Among the linkers studied, the hydrazide–hydrazone group, followed by the imine moiety, contributed most to enhanced activity. Activity was also favored by an (hetero)aromatic substituent R, although hydrazones bearing (cyclo)alkyls also exhibited some activity. The most active analogues showed MIC values as low as 0.49 µM, selective antifungal activity and no cytotoxicity. Evaluation against clinical isolates and detailed characterization of antifungal activity are ongoing.

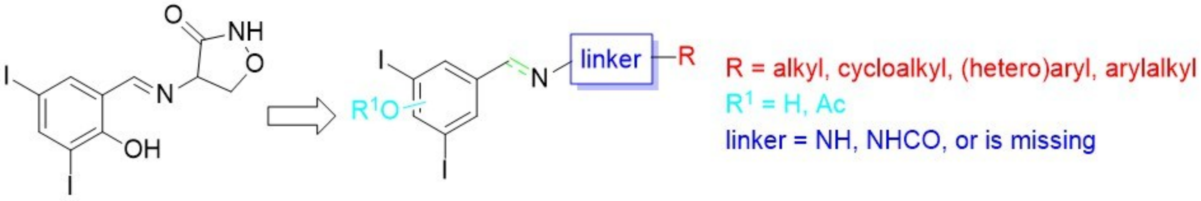

Figure 1. Investigated chemical space of iodinated antifungal agents.

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Thymosin β4 as a Metal-Responsive Regulator of Ferroptosis and Cellular Stress: Implications for Medicinal Chemistry

Joanna Izabela Lachowicz[1,2]

1. Department of Environmental Health, Occupational Medicine and Epidemiology, Wroclaw Medical University, Mikulicza-Radeckiego 7, 50-368 Wroclaw, PL, Poland;
2. Department of Medical Science and Public Health, University of Cagliari, Cittadella Universitaria, 09042 Monserrato, CA, Italy;

joanna.lachowicz@umw.edu.pl; lachowicz@unica.it

Keywords: Ferroptosis modulators, Metal-dependent redox signaling, Peptide-based chelation strategies, Stress-adaptive cell death pathways, Bioinorganic medicinal chemistry

Thymosin β4 (Tβ4) is a highly conserved, intrinsically disordered peptide with established roles in actin dynamics, tissue regeneration, angiogenesis, inflammation, and cancer progression [1]. However, its pleiotropic biological activity cannot be fully explained by G-actin sequestration alone. Here, we propose an integrated mechanistic model in which Tβ4 functions as a metal-responsive regulator of cellular stress and ferroptosis, with direct relevance for medicinal chemistry. Using NMR spectroscopy, molecular modeling, and cellular assays, we demonstrate that Tβ4 coordinates essential metal ions—including Fe^{2+}/Fe^{3+}, Zn^{2+}, and Ca^{2+}—via multiple low-affinity, spatially distributed binding sites [1–3]. Structural analyses reveal that metal coordination does not induce major secondary-structure rearrangements, consistent with the intrinsically disordered nature of the peptide [2]. Functionally, Tβ4 acts as an endogenous iron chelator, forming dynamic complexes that modulate iron availability and redox balance under stress conditions [1]. In macrophage models, extracellular Tβ4 suppresses erastin- and glutamate-induced ferroptosis, reverses mitochondrial ultrastructural damage, and restores cellular organization [1]. At the molecular level, Tβ4 counteracts ferroptotic cell death by upregulating oxidative-stress response genes (HO-1, HSP70, BAX, TXNRD1) that are otherwise suppressed during lipid peroxidation-driven toxicity [1]. Its anti-ferroptotic efficacy parallels that of reference ferroptosis inhibitors, suggesting a shared metal-dependent mechanism rather than a purely antioxidant effect. We further show that nutrient starvation profoundly alters Tβ4 trafficking, promoting the cellular uptake of extracellular Tβ4 and its Ca^{2+} complexes [3]. Under these conditions, Ca^{2+}/Tβ4 complexes are internalized into the cytoplasm, increasing intracellular calcium availability and potentially activating migration- and survival-associated signaling pathways relevant to early metastatic events [3]. Complementary zinc-coordination studies support an additional role for Tβ4 in intracellular metal buffering and redox modulation during inflammation and cellular stress [2]. Collectively, these findings establish Tβ4 as a multifunctional, redox-active metallopeptide that integrates metal coordination, ferroptosis regulation, and stress adaptation. From a medicinal chemistry perspective, Tβ4 provides a biological blueprint for designing non-toxic, peptide-based metal modulators, opening new strategies for targeting ferroptosis and metal-driven pathologies in cancer, neurodegeneration, and tissue injury

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Characterization Of Human Thymidylate Synthase Dimer Disruptor Interactions by Photoaffinity Labeling

La Gatta Valeria[1], Tagliazucchi Lorenzo[2], Aiello Daniele[1], Illuminati Davide[3], Pacifico Salvatore[3], Guerrini Remo[3], and Costi Maria Paola[1]

1. Department of Life Science, University of Modena and Reggio Emilia;
2. Department of Biotechnology, Chemistry and Pharmacy, University of Parma;
3. University of Ferrara.

valeria.lagatta@unimore.it

Keywords: thymidylate synthase, dimer disruptors, photoaffinity labeling, and proteomics

Human thymidylate synthase (hTS) is an essential enzyme in de novo DNA biosynthesis and a validated anticancer target. hTS exists in a dynamic equilibrium between an active dimer and an inactive monomer involved in autoregulatory control of protein expression [1]. Conventional active-site inhibitors induce hTS overexpression and drug resistance [1,2]. To overcome these limitations, we developed small molecules targeting the hTS dimer interface, acting as dimer destabilizers that inhibit enzymatic activity while preserving regulatory functions [2]. To investigate ligand-protein interactions and assess selectivity, a photoaffinity labeling–mass spectrometry (PAL-MS) strategy was established. PAL employs photoreactive probes containing diazirine groups that, upon UV irradiation, generate reactive intermediates able to covalently trap proximal residues. This approach enables investigation of non-covalent and transient interactions, particularly suitable for interface-targeting ligands. The incorporation of an alkyne handle further allows bioorthogonal conjugation with reporter tags via copper-catalyzed azide–alkyne cycloaddition, enabling enrichment and identification of labeled proteins by LC–MS [3]. Among the interface-targeting compounds previously developed in our laboratory, A40 was selected as a scaffold for probe design due to its favorable solubility and synthetic accessibility. A diazirine-containing analogue was synthesized and successfully applied in PAL experiments on recombinant hTS, enabling mapping of the binding region at the dimer interface. To extend it to a cellular context, alkyne-functionalized derivatives were designed. A series of pre-probe analogues was prepared to mimic the steric and electronic properties of the final constructs. Among these, A40_3pre retained the highest inhibitory activity and was selected as precursor for the development of a fully functionalized probe suitable for cellular PAL-MS studies. This work establishes an integrated medicinal chemistry and chemical biology platform for the investigation of hTS dimer destabilizers. By combining rational probe design with PAL-MS, this approach enables characterization of ligand–protein interactions at the dimer interface and supports proteome-wide selectivity assessment, providing a foundation for next-generation anticancer agents targeting thymidylate synthase.

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A Bayesian-optimized, shape-driven oneOPES framework for resolving membrane-dependent nucleation landscapes of human IAPP toward mechanism-guided design

Laus Antonio [1], Ferro Elsi [1], Atzeni Rossano [1], Pieroni Enrico [1], Valentini Maria [1], Massimo Pisu [1]
1. CRS4 (Center for Advanced Studies, Research and Development in Sardinia), Pula, Italy

laus@crs4.it

Keywords: hIAPP, amyloid aggregation, enhanced sampling, machine learning, drug discovery

Human islet amyloid polypeptide (hIAPP) aggregation is a key molecular event in type 2 diabetes. Yet early nucleation remains difficult to characterize due to heterogeneous conformational ensembles and metastable intermediates, further modulated by membrane proximity. To address this problem, we developed an enhanced-sampling framework based on oneOPES (On-the-fly Probability Enhanced Sampling) to reconstruct the free-energy landscape of hIAPP nucleation in membrane-near and membrane-far conditions [1].

The method is centered on a custom collective variable (CV) designed to encode peptide shape and conformational identity. This variable is built from structurally interpretable descriptors, including hydrogen-bond patterns and side-chain contacts, selected across reference basins spanning beta-like, alpha-like, and disordered states. The descriptor space is then compressed into a one-dimensional discriminant through an HLDA-based formulation further refined by state-specific gating terms, improving local state resolution while reducing degeneracy along the projected coordinate.

To increase robustness and transferability, collective-variable construction is coupled to an AI-assisted Bayesian hyperparameter optimization strategy, in which weights, cutoffs, and state-specific thresholds are tuned through a multi-rung workflow with progressively stricter selection criteria. This enables the systematic identification of a balanced and physically meaningful CV that preserves structural interpretability while maximizing discrimination among nucleation-relevant metastable states.

We aim to obtain a reliable free-energy landscape describing how hIAPP explores distinct metastable regions in membrane-near and membrane-far conditions, and to identify the key intermediates and barrier regions controlling the onset of aggregation [2]. These metastable states act as mechanistic gatekeepers for rational modulation of nucleation pathways, since selectively stabilizing off-pathway states or destabilizing nucleation-competent intermediates may reshape the associated free-energy barriers. Overall, this work establishes a computational framework that links advanced CV engineering, AI-assisted Bayesian optimization, and enhanced sampling to the mechanistic dissection of hIAPP nucleation and to the identification of structurally actionable states for anti-aggregation design [3].

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From Fragments to Leads: Design and Synthesis of High-Affinity Sirtuin 6 Inhibitors via Structure-Guided Optimization

Andrea Mancini, [1] Emanuele Fabbrizi,[1] Weijie You, [2] Wei Zheng, [3,4] Francesco Fiorentino, [1] Daniela Trisciuoglio, [5] Francesco Spallotta, [6] Clemens Steegborn, [2] Dante Rotili, [7] Antonello Mai.[1]

1. Department of Drug Chemistry and Technologies, Sapienza University of Rome, Rome, Italy
2. Department of Biochemistry, University of Bayreuth, 95445 Bayreuth, Germany
3. Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
4. Geriatric Research, Education, and Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, USA;
5. Institute of Molecular Biology and Pathology (IBPM-CNR), Rome, Italy
6. Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Italy;
7. Department of Sciences, Roma Tre University, Rome, Italy

an.mancini@uniroma1.it

Keywords: Epigenetics, Sirtuins, Cancer

As an important NAD$^+$-dependent enzyme, SIRT6 has received significant attention since its discovery. Recent evidence has demonstrated that SIRT6 functions as a Lys deacetylase, deacylase, and mono-ADP-ribosyltransferase, participating in variety of cellular signaling pathways and regulating a wide range of physiological and pathological processes. Specifically, 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-dependent manner, with mounting evidence regarding its role in tumor initiation and progression. Given these key roles in various cancer types, we set out to develop novel SIRT6 inhibitors that may lay the ground for new anticancer drugs. After screening a fragment library using protein crystallography, we 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; the resulting lead, compound S6023, showed an IC$_{50}$ = 5 µM. This compound selectively inhibits SIRT6 and is active in W138 cells, where it increases levels of the senescence markers p21 and γH2AX. To improve the potency of S6023, we developed over thirty analogues. Among these, we identified MC4637, which possesses a similar binding mode to S6023 according to its co-crystal structure with SIRT6. Notably, in the fluorogenic enzymatic FdL assay, MC4637 exhibited an IC$_{50}$ = 600 nM and did not significantly affect the activity of other SIRT isoforms (Sirt1-3 and Sirt5) even at the highest concentration tested (25 µM). The anticancer activity of these SIRT6 inhibitors was evaluated across a representative panel of human cancer cell lines, such as H1299, A549, and HT29. MC4637 emerged as the most potent inhibitor, displaying single digit micromolar IC$_{50}$ values after 48 h of treatment, consistent with its superior enzymatic potency. In pancreatic ductal adenocarcinoma (PDAC) models (BxPC-3 and Mia-PaCa-2), a dose-dependent reduction in proliferation was observed. BxPC-3 cells were particularly sensitive (1 µM), whereas Mia-PaCa-2 cells required higher concentrations (5 µM), reflecting genetic differences such as KRAS mutational status. The inactive analogue MC4692 showed no effect, confirming the specificity of MC4637.

Deciphering the impact of phosphorylation on HuR functional domains through molecular dynamics simulations

Marchese Emanuela[1], Paravati Maria Rosaria [1], Ambrosio Francesca Alessandra [1], Castoria Gabriella [2], Tesei Anna [3], Costa Giosuè [1], Collina Simona [4], and Alcaro Stefano [1,5]

1. Dipartimento di Scienze della Salute, Università degli Studi "Magna Græcia" di Catanzaro, Campus "S. Venuta", Viale Europa, 88100, Catanzaro, Italy;
2. Dipartimento di Medicina di Precisione, Università della Campania "L. Vanvitelli", Naples, Italy;
3. Biosciences Laboratory, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) "Dino Amadori", I-47014 Meldola, Italy;
4. Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy;
5. Associazione CRISEA - Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, località Condoleo di Belcastro Catanzaro, Italy;

e.marchese@unicz.it

Keywords: human antigen R, phosphorylation, molecular dynamics simulations, and anticancer.

RNA-binding proteins (RBPs) are key regulators of post-transcriptional gene expression and promising therapeutic targets in several pathologies [1]. Human antigen R (HuR, encoded by *ELAVL1*) is a well-characterized RBP that modulates the stability and translation of mRNAs involved in oncogenesis and inflammation. HuR activity is tightly controlled by post-translational modifications, particularly phosphorylation, which affects RNA-binding affinity and subcellular localization. Specifically, phosphorylation sites located within the RRM1 and RRM2 domains (S88, S100, T118) have been shown to directly influence HuR interaction with target transcripts, promoting dissociation from specific mRNAs and thereby modulating the stability of transcripts involved in proliferation and survival pathways. In contrast, phosphorylation events in the RRM3 domain and hinge region (S221, S318) regulate HuR nucleocytoplasmic shuttling [2].

Despite these insights, the molecular mechanisms by which site-specific phosphorylation alters HuR's conformational dynamics and RNA recognition remain poorly understood. Here, we first employed GRID-based interaction energy studies to map favorable phosphorylation sites within HuR functional domains [3]. This mapping guided subsequent comparative MD simulations, performed to investigate the structural effects of phosphorylation on HuR variants [4]. By characterizing the protein–RNA interface and global flexibility, we aim to provide a mechanistic basis for how these modifications modulate HuR function. This work could establish a structural framework for the rational design of selective HuR modulators, offering new avenues for therapeutic intervention in oncology.

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Substituted Cinnamylpiperazines as Modulators of AcrAB-TolC-Mediated Antibiotic Resistance

Mezeiova Eva[1], Kufa Martin [1,2], Hympanova Michaela[1], Korabecny Jan[1] and Roh Jaroslav[2]

1. Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 50005, Hradec Kralove, The Czech Republic;
2. Faculty of Pharmacy in Hradec Kralove, Charles University, Akademika Heyrovskeho 1203, 50005, Hradec Kralove, The Czech Republic.

eva.mezeiova@fnhk.cz

Keywords: antibiotic resistance, AcrAB-TolC efflux pump, efflux pump inhibitors, cinnamylpiperazine derivatives

The rapid spread of antibiotic resistance represents a major threat to effective antimicrobial therapy worldwide. In Gram-negative bacteria, resistance is frequently mediated by multidrug efflux systems that actively expel antibiotics from the cell, thereby reducing intracellular drug exposure and treatment efficacy. Among these systems, the AcrAB-TolC efflux pump of *Escherichia coli* is one of the most prominent and extensively investigated resistance determinants. In the present work, we describe the design, synthesis, and biological evaluation of a series of substituted cinnamylpiperazine derivatives as potential efflux pump inhibitors targeting the AcrAB-TolC system.[1] The ability of the compounds to restore antibiotic activity was assessed using both wild-type *E. coli* strains and strains deficient in efflux pump function, allowing mechanistic insight into their mode of action. The observed activity profiles strongly support AcrAB-TolC as the primary target of the investigated molecules.[2] One representative compound, K2855, demonstrated pronounced synergistic effects in combination with selected antibiotics, yielding fractional inhibitory concentration indices of 0.33 with linezolid and 0.31 with erythromycin in checkerboard assays. These results are comparable to those obtained with the reference inhibitor phenylalanine-arginine β-naphthylamide and exceed the performance of the structurally related inhibitor 1-(1-naphthylmethyl)piperazine. Taken together, our findings identify substituted cinnamylpiperazines as a promising chemical class for efflux pump inhibition and highlight their potential to counteract bacterial resistance by enhancing the efficacy of existing antibiotics. Further studies are underway to refine structure–activity relationships and to improve the pharmacokinetic and pharmacodynamic properties of this scaffold.

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Nanostructured Liposomal Systems for Safer Delivery of Triphenylphosphonium Derivatives

Joana Moreira[1], Clarisse Lopes[1], Barbara Nunes[2,3], Cláudia Sampaio Machado[1], Rita Cardoso[1], Fernando Cagide[1] and Carlos Fernandes[1]

1. RISE-Health, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal;
2. LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering (FEUP), University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;
3. ALiCE—Associate Laboratory in Chemical Engineering, FEUP, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Keywords: Quaternary phosphonium compounds, Liposomes, *In vitro* toxicity assessment

Quaternary phosphonium compounds, particularly triphenylphosphonium salt (TPPs) derivatives, exhibit antimicrobial and antifungal activity against resistant strains [1]. However, their cytotoxicity toward human cells limits clinical application, highlighting the need for effective delivery systems [1]. Nanocarriers offer a strategy to control release and improve safety, with liposomal incorporation potentially reducing off-target toxicity [2].

Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes loaded with TPPs derivatives presenting different alkyl chain length (Cx-TPPs; x=8, 10, 12, 14 and 16 carbons) were prepared via thin-film hydration.[3] Particle size and zeta potential were measured by dynamic light scattering, while encapsulation efficiency was quantified via ultra-high-performance liquid chromatography. Storage stability, pH responsiveness, and mucin interactions were also evaluated. The toxicity of the nanoformulations was assessed in Normal Human Dermal Fibroblasts and Human Hepatocellular Carcinoma cell lines using resazurin and sulforhodamine B assays and compared with free Cx-TPPs.

The encapsulation of Cx-TPP derivatives was successfully achieved and resulting nanoformulations showed sizes below 300 nm with surface charge dependent on alkyl chain length, and a good storage stability for up to 8 weeks. Nanoformulations showed interactions with mucins and improved stability under mildly acidic to neutral pH conditions. The most promising nanoformulations showed reduced cytotoxicity when compared with free Cx-TPP in both cell lines. Overall, DPPC liposomes loaded with Cx-TPP derivatives displayed suitable physicochemical properties and reduced cytotoxicity, supporting their potential as safer antimicrobial nanocarriers.

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Replacement of the Nitrile with Boronic Acid in Second-Generation Anti-androgen Enzalutamide: Design and Synthesis

Needle Adam A.[1], Doležal Martin[1], and Kučerová-Chlupáčová Marta[1]

1. Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Akademika Heyrovského 1203, 500 03 Hradec Králové, Czech Republic

needlea@faf.cuni.cz

Keywords: *nonsteroidal anti-androgen; enzalutamide; castration-resistant prostate cancer; boronic acid*

Castration resistant prostate cancer, which does not respond to second generation nonsteroidal anti-androgen treatment, is putting pressure on the development of new agents that will antagonise mutant androgen receptor variants and inhibit the binding of male sex hormones [1]. A recent publication by our team has revealed that it is possible to replace the nitro group in the structure of flutamide with a boronic acid functional group [2]. In the structures of second-generation nonsteroidal anti-androgens, the nitrile functional group replaces the original nitro group. We have replaced the nitrile group in enzalutamide analogues with a boronic acid residue that contains two oxygen atoms, which could mimic the nitro group in the flutamide molecule. The initial idea was that boron can form a dative bond with biological nucleophiles in amino acid residues [3]. Preliminary *in silico* studies have shown a hydrogen bond between Met745 and the boronic acid moiety instead of the proposed Arg752. Therefore, non-covalent binding modes may also provide promising molecules. This could lead to the discovery of an agent effective for the treatment of castration-resistant prostate cancer. The effect of structural modifications on antiandrogenic activity is being studied by introducing various hydrophilic, lipophilic, electron-withdrawing, and electron-donating groups into the structure. Variation of the halogen adjacent to the boronic acid moiety is also examined. Our five-step synthesis process has been successfully optimised and can be utilised in the synthesis of further series. The first series is being evaluated in the LAPC-4 androgen dependent human prostate cancer cell line and in the PC-3 androgen independent human prostate adenocarcinoma cell line. The selectivity of the compounds will be assessed in HepG2 and HK-2 cell lines. To confirm the interaction of the prepared ligands with the androgen receptor, a fluorescence polarisation-based competition binding assay is planned.

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Novel Thiazolidinone-Isatin Hybrids as Potent hCA IX Inhibitors Enhance Doxorubicin-Induced Apoptosis in Hypoxic Glioblastoma Cells

Onali Alessia[1], Guarnieri Lorenza[2], Bosco Francesca[2], Lupia Antonio[1], Sanna Erica[1], Demuru Laura[1], Atzeni Giulia[1], Meleddu Rita[1], Cottiglia Filippo[1], Supuran T.Claudiu[3], Distinto Simona[1], Maccioni Elias[1]

1. Department of Life and Environmental Sciences, University of Cagliari, 09042 Monserrato, Italy
2. Section of Pharmacology, Department of Health Sciences, University "Magna Graecia" of Catanzaro, 88100 Catanzaro, Italy
3. Dipartimento NEUROFARBA, Sezione di Scienze Farmaceutiche, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy

Carbonic anhydrase IX (hCA IX) is a HIF-1α-regulated transmembrane zinc-metalloenzyme overexpressed in hypoxic solid tumors, where it promotes extracellular acidification, invasive migration, and chemoresistance [1]. Its overexpression in glioblastoma multiforme (GBM) correlates with poor prognosis [2,3], yet no hCA IX inhibitor has reached clinical approval, underscoring the need for potent, isoform-selective compounds. Applying a molecular hybridization strategy based on the tail approach, we designed and synthesized a series of thiazolidinone-isatin hybrids (3a-i, 4a-i) featuring a benzenesulfonamide zinc-binding group coupled to an extended tail projected into the mid-cavity and entrance regions of the hCA IX active site. Enzymatic assays identified compound **3f** as the most potent and selective hCA IX inhibitor (Ki = 8.6 nM; SI hCAI/IX = 631; SI hCAII/IX = 32.7). N-acetylation of the sulfonamide nitrogen completely abolished activity across all derivatives (Ki > 10,000 nM), confirming the free -SO_2NH_2 as strictly required for Zn^{2+} coordination. Molecular docking confirmed coordination of the catalytic zinc ion and key hydrogen bond interactions with Gln92, Thr199, and Thr200. In T98G glioblastoma cells, 3f (5 μM, non-cytotoxic as single agent) significantly sensitized cells to doxorubicin under both normoxic and chemically-induced hypoxic conditions ($CoCl_2$), reducing viability from ~76% to ~39%. Western blot analysis confirmed activation of the intrinsic apoptotic pathway, evidenced by increased Bax/Bcl-2 and cleaved caspase-3 ratios. These findings establish 3f as a promising lead for CA IX-targeted antitumor strategies in chemoresistant glioblastoma.

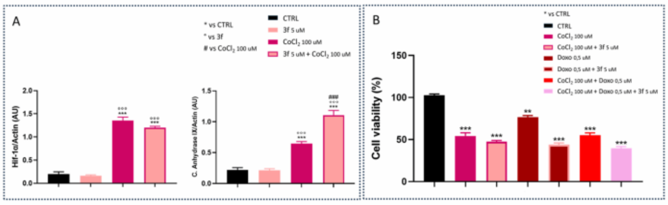

Figure 1. Hypoxia model validation and antiproliferative activity of 3f in T98G glioblastoma cells. Data are expressed as mean ± SEM. **p < 0.01, ***p < 0.001 vs CTRL.

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From Viral Proteins to Host Targets: Unveiling Casein Kinase 2 as the Antiviral Mechanism of the [4,7]-Phenanthroline Scaffold

Paravati Maria Rosaria[1], Romeo Isabella [1], Carta Antonio [2], Deiana Andrea [2], Murineddu Gabriele [2] and Alcaro Stefano [1]

1. Dipartimento di Scienze della Salute, Università “Magna Græcia” di Catanzaro, Campus “S. Venuta”, Viale Europa, 88100 Catanzaro, Italy;
2. Department of Medicine, Surgery and Pharmacy, University of Sassari, Via Muroni 23a, Sassari, Italy;

mrparavati@unicz.it

Keywords: *broad-spectrum antivirals, host-targeting, Structural-Based Drug Design, phenanthroline scaffold, casein kinase 2.*

The [4,7]-phenanthroline compound PS1100 exhibits broad-spectrum activity against several viruses, including SARS-CoV-2 and Zika, making it a compelling scaffold for pan-viral drug discovery [1]. Initially, a computational target fishing study was performed on the key non-structural proteins (NSPs) of SARS-CoV-2 (NSP3, 5, 12, 13, 14, 15, and 16) [2]. However, molecular docking and Molecular Mechanics Generalized Born Surface Area (MM/GBSA) analyses indicated poor affinity for these viral enzymes, a finding corroborated by *in vivo* assays showing no direct antiviral enzymatic inhibition [3]. This negative outcome prompted a shift toward host-directed therapy, hypothesizing that PS1100 targets cellular factors exploited by viruses, such as Casein Kinase 2 (CK2) [4]. CK2 is a ubiquitous Ser/Thr kinase essential for the replication of numerous viruses. It exists as a heterotetramer with two catalytic (α or α’) and two regulatory (β) subunits, featuring a conserved ATP-binding site and a specific αD pocket [5]. Given its structural similarity to silmitasertib, we investigated PS1100 as a CK2 inhibitor. Our *in silico* studies showed binding affinities comparable or superior to silmitasertib for the α and α’ subunits, respectively [3]. Thermal Shift Assay (TSA) demonstrated a modest binding affinity, while X-ray crystallography definitively confirmed that PS1100 occupies the CK2 binding pocket. Subsequently, building on these results, a library of 1,945 phenanthroline derivatives was designed, identifying four lead candidates currently under *in vitro* evaluation. These were used to develop a second library of 266 bivalent molecules, designed to simultaneously target the ATP site and the αD pocket via 5-8 carbon alkyl linkers, inspired by the inhibitor KN2. Ongoing *in silico* studies are evaluating their binding modes, supporting PS1100 derivatives as promising agents for host-directed, broad-spectrum antiviral strategies.

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Nanocarrier-based delivery of 20-hydroxyecdysone modulates IL-17/IL-23 signaling in psoriasis models

Piwowarczyk Ludwika[1], Majchrzak-Celińska Aleksandra[2], Kleszcz Robert[2], Musielak Ewelina[2], Gajewska Julia[2], Szkudlarek Jagoda[1], Czerniel Joanna[1], and Krajka-Kuźniak Violetta[2]

1. Poznan University of Medical Sciences, Department of Pharmaceutical Chemistry, Rokietnicka 3, Poznań, Poland
2. Poznan University of Medical Sciences, Department of Pharmaceutical Biochemistry, Rokietnicka 3, Poznań, Poland

lpiwowarczyk@mail.com

Keywords: psoriasis, 20-hydroxyecdysone, lipidnanocarriers, interleukins

Psoriasis is a chronic inflammatory skin disease characterized by dysregulated immune responses and epidermal hyperproliferation, in which cytokine networks involving IL-17 and IL-23 play a central pathogenic role [1]. Keratinocytes actively contribute to this process by producing pro-inflammatory mediators and sustaining local immune activation. Despite advances in systemic therapies, effective topical strategies targeting these pathways remain limited due to the impaired barrier function of psoriatic skin [2]. 20-Hydroxyecdysone (20-HE), a natural phytoecdysteroid, exhibits anti-inflammatory, antioxidant, and cytoprotective properties, but has limited skin penetration and bioavailability. Therefore, lipid-based nanocarrier delivery systems have been developed to enhance dermal delivery and improve the biological activity of active compounds [3]. In this study, lipid-based nanocarrier formulations containing 20-HE were developed and characterized, and their biological activity was evaluated in human epidermal keratinocytes (HEKs) and psoriasis-derived keratinocytes (PHEKs). Cells were treated with the respective nanoformulations for 48 hours. Cytokine levels, including IL-17A and IL-23, were quantified using a Western blot assay. All tested nanoformulations of 20-HE modulated inflammatory responses in keratinocytes, with differential effects depending on the carrier type. In conclusion, nanoformulations containing 20-HE exhibit distinct anti-inflammatory profiles in psoriatic keratinocytes. Modulation of IL-17/IL-23-associated signaling highlights the potential of these nanocarrier systems to become promising candidates for topical psoriasis therapy.

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Combining DHODH inhibitors and pronucleotides to block viral replication

Lara Prilop[1], Allison Groseth [2], Thomas Hoenen [2], and Chris Meier [1]

1. Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany;
2. Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Südufer 10, D-17493 Greifswald-Insel Riems, Germany;

lara.prilop@uni-hamburg.de

Keywords: Dihydroorotate dehydrogenase, viral RdRp, haemorrhagic fever virus, Pronucleotides

Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme involved in the de novo pyrimidine synthesis, leading to the formation of uridine monophosphate (UMP). UMP is further converted to other pyrimidine nucleotides needed for the biosynthesis of RNA and DNA. Blocking DHODH with small synthetic molecules should result in a depletion of pyrimidines in the nucleotide pool.[1] In combination therapy, DHODH inhibitors can enhance the effectiveness of viral RNA-polymerase targeting nucleoside analogs by reducing the competition with cellular pyrimidine nucleotides for their incorporation into the viral RNA.[2] For antiviral activity, these compounds must be converted into their triphosphate form, which serves as the final metabolite that inhibits the viral polymerase. To improve bioavailability and bypass phosphorylation steps, prodrug strategies have been developed by our research group[3].

Herein, we describe structural optimizations of the lead structure of a DHODH inhibitor, focusing on improving membrane permeability while maintaining antiviral efficacy. Additionally, the synthesis of 4'-fluorouridine, an antiviral pyrimidine nucleoside analog, and its conversion into a pronucleotide using the Tri*PPP*ro approach will be described.

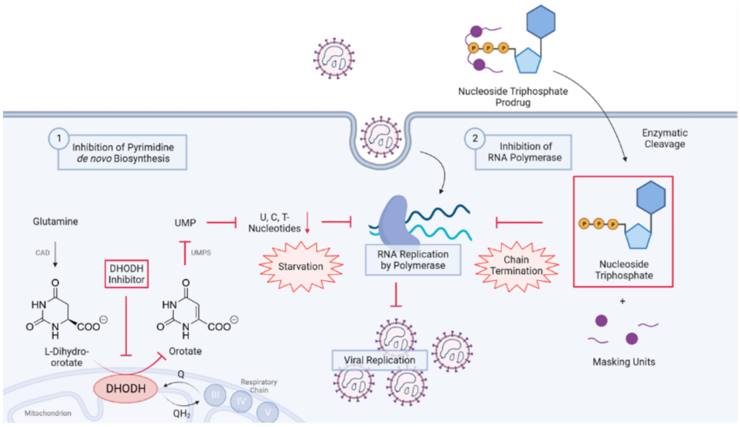

Figure 1. Combinational approach involving DHODH inhibitors and pronucleotides

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Balancing the potency and toxicity of novel antimicrobial agents by rational design

Rocha Teresa[1], Nunes Bárbara[2,3], Simões Manuel[2,3], Fernandes Carlos[1], Borges Fernanda[1], Cagide Fernando[1]

1. RISE-Health, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto;
2. LEPABE – Laboratory for Process Engineering, Biotechnology and Energy, Faculty of Engineering, University of Porto;
3. ALiCE–Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto.

up202005935@edu.fc.up.pt

Keywords: *Antimicrobial resistance, fluorinated compounds, antibacterial activity, cytotoxicity*

Antimicrobial resistance (AMR) is one of the greatest threats facing human health worldwide, as drug-resistant pathogens increasingly compromise available treatment options. Despite the recognised urgency, only a few novel antibiotic classes have reached the clinic in recent decades, highlighting the critical need for novel antimicrobial agents. Quaternary ammonium (QAS) and quaternary phosphonium (QPS) salts have emerged as promising candidates due to their broad-spectrum activity and tunable molecular scaffold. Previous studies demonstrated the potent antimicrobial efficacy of dodecyl and tetradecyl triphenylphosphonium (TPP) derivatives against *S. aureus*, *A. baumannii*, *E. coli*, and *C. albicans*. However, significant cytotoxic and hemolytic effects were reported, limiting their therapeutic potential [1,2]. To address this, two rational design strategies were pursued: (i) conjugation of the TPP phenyl rings with fatty acid chains, and (ii) change of the phosphonium headgroup with QAS bearing electron-withdrawing groups. A small library of fluorinated pyridinium, isoquinolinium, quinolinium, and methylpiperidinium salts with dodecyl and tetradecyl chains, and TPP-fatty acid conjugates, was synthesised. Antimicrobial activity was assessed against an antibiotic-susceptible *S. aureus* strain and extended to a clinically relevant panel of Gram-positive, Gram-negative bacteria, and fungi (*C. albicans*, *C. neoformans*). Cytotoxicity was evaluated in HepG2 and NHDF cell lines, and mitochondrial membrane polarisation and mitochondrial mass were assessed. All compounds inhibited *S. aureus* growth within the same low micromolar range as TPP analogues. Six showed relevant broad-spectrum antibacterial and/or antifungal activities. Based on activity and chemical diversity, a subset of compounds was selected for cytotoxicity evaluation, revealing a significantly improved profile relative to TPP analogues. Mitochondrial data confirmed that toxicity was dependent on mitochondrial function. This work provides a rational framework to optimise fluorinated QAS-based antimicrobial agents enhancing their potency while reducing cell host toxicity.

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The Coronavirus Helicase as a Drug Target: New Chemical Approaches Against a Conserved Viral Engine

Sanna Erica[1], Corona Angela[1], Lupia Antonio[1], Onali Alessia[1], Atzenia Giulia1, Demuru Laura[1], Meleddu Rita[1], Cottiglia Filippo[1], Distinto Simona[1], Tramontano Enzo[1], Maccioni Elias[1]

1.1 Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, S.P. 8 km 0.700, 09042, Cagliari, Italy

erica.sanna@unica.it

Helicase of human coronaviruses (hCoVs) is responsible for the RNA unwinding in a 5’ to 3’ direction, a process driven by the ATP hydrolysis. Given its crucial role in viral replication and its highly conserved sequence among hCoVs, this enzyme represents a promising and attractive target for the development of new antiviral drugs.[1]
In this study, with the goal to identify potential Pan-hCoVs helicase inhibitors, we have rationally designed and synthesized a library of compounds. These compounds were characterized using structural (single-crystal X-ray diffraction) and spectroscopic (NMR, MS) techniques and subsequently, evaluated for their biological activity. Our results revealed that all compounds were able to inhibit both SARS-CoV-2 helicase-associated enzyme activities, namely NTPase and unwinding activity, showing IC50 values in the low micromolar range. Among them, several compounds also demonstrated potent antiviral effects, significantly inhibiting SARS-CoV-2 replication at low EC50 values, without causing notable cytotoxicity (CC50).
Furthermore, some of the most promising candidates demonstrated antiviral activity against other human coronaviruses such as MERS-CoV and hCoV-229E, highlighting their potential as pan-hCoV inhibitors.
These findings underscore the potential of targeting hCoV helicase as a strategy for developing effective treatments against SARS-CoV-2 and other hCoVs, opening the door to the development of novel therapeutic agents that could address both current and future emerging coronavirus-related diseases.

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Novel tricyclic flavonoids as promising anti-MRSA agents

Laura Gabriela Sarbu

1. Department of Chemistry, “Alexandru Ioan Cuza” University of Iasi, Romania

laura.sarbu@uaic.ro

Keywords: *synthetic flavonoids, benzopyran, new anti-MRSA agents, bacteriostatic, bactericidal*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered the main cause of nosocomial and community-associated infections. Because of the antimicrobial resistance, MRSA infections are difficult or impossible to treat, leading to high mortality rates and significant economic and societal costs [1]. In view of the MRSA challenge to public health all over the world, identification of new effective anti-MRSA agents is a high medical priority. In our study, a new series of tricyclic flavonoids with a methyl substituent on ring A of the flavonoid skeleton has been synthesized to assess their antimicrobial properties. The structures of novel synthetic tricyclic flavonoids and of their 3-dithiocarbamic flavanones have been proved by X-ray structural analyses. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were used to evaluate the antimicrobial activity. Growth kinetics and time kill assays were employed to confirm the antibacterial effectiveness. Our results showed that the tricyclic flavonoids exhibited important antibacterial and antifungal activity, with MIC and MBC values as low as 1.95 µg/mL and 3.90 µg/mL recorded for compound **1** against a multidrug resistant MRSA strain [2]. Flavonoid **1** induced a more important bacteriostatic effect compared with chloramphenicol, inhibiting the bacterial growth for up to 24 h at concentrations equivalent to 2 × MIC. Also, **1** exhibited a significant bactericidal activity, with no viable cells evidenced after 6 h of incubation in the presence of MBC and a total kill effect recorded up to 24 h. All the data support the idea that flavonoid **1** is a reliable candidate to develop effective anti-MRSA agents.

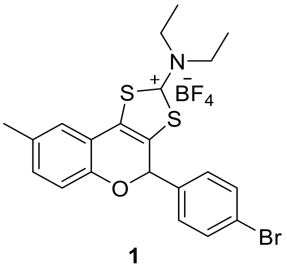

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Figure 1. The structure of tricyclic synthetic flavonoid **1**

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DISCOVERY OF NOVEL FLT3-TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

Sarova Denisa[1,2], Gorecki Lukas[2], Krystof Vladimir[3], Rezacova Martina[4], Ceckova Martina[5], Korabecny Jan[2], and Kratky Martin[1]

3. Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University, Akademika Heyrovskeho 1203, 500 03 Hradec Kralove, Czechia
4. Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czechia
5. Laboratory of Growth Regulators, Palacky University and Institute of Experimental Botany, The Czech Academy of Sciences, Slechtitelu 27, 783 71 Olomouc, Czechia
6. Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University, Simkova 870, 500 03 Hradec Kralove, Czechia
7. Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Akademika Heyrovskeho 1203, 500 03 Hradec Kralove, Czechia

sarovad@faf.cuni.cz

Keywords: *cancer, FLT3, medicinal chemistry, acute myeloid leukemia,* and *drug development*

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy that continues to be associated with poor therapeutic outcomes. While conventional chemotherapy remains the cornerstone of AML treatment, increasing attention has been directed toward targeted therapies that exploit specific genetic abnormalities in leukemic cells. One of the most common oncogenic drivers in AML is the FLT3 internal tandem duplication (FLT3-ITD) mutation, which results in constitutive activation of the FLT3 receptor and drives uncontrolled cellular proliferation. Although FLT3 inhibitors such as midostaurin and gilteritinib have been approved for clinical use, their effectiveness is often limited by the development of drug resistance, highlighting the need for next-generation inhibitors with improved potency and resistance profiles.[1]

To address this need, we identified a novel series of small-molecule FLT3 inhibitors based on our lead compound K1872. Structure-guided optimization generated several distinct compound series and produced several candidates with nanomolar activity against FLT3-ITD as well as strong antiproliferative effects in AML cell lines.[2] Notably, the most advanced compounds exhibited strong kinase selectivity relative to clinically established FLT3 inhibitors. Their antileukemic efficacy was further confirmed in primary leukemic blasts from AML patients, supporting the translational potential of this scaffold for the development of next-generation FLT3-targeted therapies.

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Deep Eutectic Solvents (DES) for the Sustainable Extraction of Phytocompounds from *Cannabis sativa*

Scarpelli Rosa[1,2], Gualtieri Alessandro[1,2], Frisina Marialaura[1,2], David Maria Teresa[2], Procopio Antonio[1,2], Nardi Monica[1,2]

1. Departement of Health Sciences, University Magna Graecia of Catanzaro, Campus S. Venuta, Viale Europa, Germaneto, 88100 Catanzaro (Italy)
2. "AGreenFood" Research Center, University "Magna Græcia" of Catanzaro, Campus Universitario "S Venuta", 88100 Catanzaro (Italy)

rosa.scarpelli@studenti.unicz.it

Keywords: Deep eutectic solvents, cannabinoids, eco-sustainable extraction, CBD.

Cannabidiol (CBD) is one of the most extensively studied phytocannabinoids due to its lack of psychoactive effects and its proven biological properties [1], including anti-inflammatory, anxiolytic and neuroprotective effects. Currently, purified CBD formulations are already usedin the clinical treatment of drug-resistant epilepsy. However, conventionalextraction methods based on organic solvents present issues related to toxicity, flammability and high energy and environmental impact [2]. This study proposes an eco-sustainable extraction of CBD from *Cannabis sativa L.*, based on the use of Deep Eutectic Solvents (DES) [3]. Subsequently,the research focused on the preparation of DES composed directlyof CBD [4] and oleic acid, exploring their potential for pharmaceutical applicationsas innovative systems in the pharmaceutical sector.

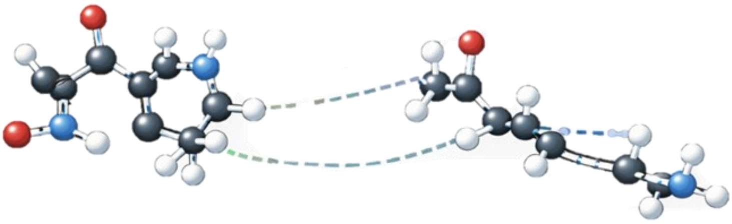

Figure 1. Schematic representation of the hydrogen-bond interaction between oleic acid and cannabidiol (CBD) during the formation of the deep eutectic solvent (DES)

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Polymerization-induced self-assembly of ROS-sensitive nanovesicles for dual-drug delivery in ALS therapy

Lisa Sequeira[1], Rita Saraiva[1], Ana Rita Alfenim[1], Joana Moreira[1], J. Eberhardt[2], Miguel Pinto[1], Johannes Brendel[2], Carlos Fernandes[1]

1. RISE-Health, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal;
2. Macromolecular Chemistry, University of Bayreuth, Universitätsstr. 30, 95447 Bayreuth, Germany.

lsequeira@med.up.pt

Keywords: *ALS, Riluzole, Rasagiline, reactive oxygen species, ROS-responsive nanovesicles*

Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disorder with a multifactorial pathophysiology characterized by an oxidative environment. Current therapies primarily focus on extending survival and slowing the progression of physical decline[1], underscoring the urgent need for neuroprotective strategies that target multiple mechanisms simultaneously. Nanotechnology-based delivery systems offer a promising approach to enhance therapeutic efficacy compared to conventional drug administration that when combined with reactive oxygen species (ROS)-sensitive moieties enable the controlled and targeted release of therapeutic agents. The combination of Riluzole (RIL), an FDA-approved drug for ALS, with Rasagiline Mesylate (RAS), which provides neuroprotective effects, represents a strategy to achieve synergistic benefits and improve treatment outcomes. To realize this potential, ROS-sensitive RIL/RAS-loaded vesicles were synthesized via polymerization-induced self-assembly (PISA). Poly(N-acryloylmorpholine) (PNAM) served as the macro chain transfer agent, while N-acryloylthiomorpholine (NAT) was used as the monomer[2]. Different PNAM/NAT ratios were tested as well as the concentration of NAT in the reactional mixture. The drug content in the vesicles was quantified using a high-performance liquid chromatography (HPLC) system, while the size and morphology of the vesicles were characterized through dynamic and electrophoretic light scattering (DLS and ELS). The study of the degradation kinetics was performed under physiological and oxidative environment conditions at 37 ºC. A preliminary evaluation of the pharmacological effect of the novel ROS-sensitive nanoformulations was performed using neuronal and nasal epithelial cell lines.

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A NOVEL FLT3-TARGETED STRATEGY TO OVERCOME DRUG RESISTANCE IN ACUTE MYELOID LEUKEMIA

Svobodova Barbora [1], Lukas Gorecki [1], Jan Korabecny [1], Martina Ceckova [2], Martina Rezacova [3]

1. Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic; jan.korabecny@fnhk.cz
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Hradec Kralove, Czech Republic; martina.ceckova@faf.cuni.cz
3. Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University, Simkova 870, 500 03, Hradec Kralove, Czech Republic; rezacovam@lfhk.cuni.cz

Keywords: *Acute myeloid leukemia, FLT3 kinase, FLT3 inhibitors, drug resistance, apoptosis*

Acute myeloid leukemia (AML) is a highly aggressive hematologic malignancy associated with poor therapeutic responses and low cure rates [1]. Despite significant advances in targeted treatment strategies, clinical outcomes for many patients remain unsatisfactory [2]. Among the molecular abnormalities implicated in AML pathogenesis, mutations in the FMS-like tyrosine kinase 3 (FLT3) gene are particularly prevalent, occurring in approximately 30% of cases and constituting a major therapeutic target. Although FLT3 inhibitors such as midostaurin and gilteritinib have entered clinical practice, the rapid development of resistance severely limits their long-term efficacy, underscoring the urgent need for novel FLT3-directed therapies [3]. Through comprehensive kinase screening, we identified a small-molecule compound, K1872, which exhibits unique structural characteristics and a pharmacophore distinct from currently available FLT3 inhibitors. Molecular dynamics simulations suggest that K1872 may effectively circumvent resistance mechanisms commonly associated with existing FLT3-targeted agents. Ongoing hit-to-lead and lead-optimization efforts are focused on evaluating the anti-proliferative and pro-apoptotic effects of K1872 derivatives in AML cell lines and patient-derived samples. Preliminary results demonstrate superior activity of K1872-related compounds in FLT3-mutated AML cells, particularly through robust induction of apoptosis, together with selective inhibition of FLT3 kinase activity. Collectively, these findings identify K1872 derivatives as promising candidates for overcoming FLT3 inhibitor resistance and improving therapeutic outcomes in AML.

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Cationic Liposomes Co-Loaded with Cannabidiol and Additional Natural Bioactive Compounds for In Vitro Evaluation Against Glioblastoma Multiforme

Szkudlarek Jagoda [1,2], Piwowarczyk Ludwika [1], Krajka-Kuzniak Violetta [3], Majchrzak-Celińska Aleksandra [3], and Jelińska Anna [1]

1. Poznan University of Medical Sciences, Chair and Department of Pharmaceutical Chemistry, Rokietnicka 3, 60-803 Poznań, Poland;
2. Doctoral School, Poznan University of Medical Sciences, 70 Bukowska, 60-812 Poznań, Poland;
3. Chair and Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, 3 Rokietnicka, 60-806 Poznań, Poland

jszkudlarek@ump.edu.pl

Keywords: *glioblastoma; liposomes; cannabidiol; natural compounds*

Glioblastoma multiforme is a highly aggressive brain tumor associated with poor clinical outcomes. Despite advances in standard therapies, treatment efficacy remains limited, prompting the search for new therapeutic strategies [1]. Natural compounds have emerged as promising candidates due to their diverse biological activities, but their clinical application is often restricted by limited bioavailability [2]. In this setting, nanocarrier-based delivery systems, particularly liposomes, offer an attractive approach by improving drug stability, enhancing bioavailability, and facilitating more effective transport of therapeutic agents to tumor tissue [3]. In this study, cationic liposomes were developed as carriers for cannabidiol (CBD) co-encapsulated with additional natural compounds for in vitro evaluation against glioma cells. The formulations were prepared by the thin-film hydration method, followed by extrusion to obtain more uniform vesicles. Physicochemical characterization included particle size, polydispersity index, and zeta potential measurements. Anticancer activity was assessed in U-138 MG cells using the MTT assay after 24 and 48 h of incubation, with non-cancer cells used as a reference. Liposomal systems showed nanoscale particle size, low polydispersity, and positive zeta potential. In vitro studies demonstrated that co-loaded formulations reduced glioma cell viability more effectively than the empty carrier, particularly after longer incubation. One of the co-loaded formulations showed a stronger antiproliferative effect in U-138 MG cells compared with the other formulation. Overall, cationic liposomes appear to be promising carriers for CBD-based combination therapy in glioblastoma and warrant further investigation.

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Hydration Medium Matters: Development and Physicochemical Evaluation of 20-Hydroxyecdysone Niosomes for Topical Psoriasis Treatment

Szkudlarek Jagoda [1,2], Anglart Gabriela [1], Szymon Tomczak [1], Dariusz T. Mlynarczyk [3], Jelińska Anna [1], and Piwowarczyk Ludwika [1]

1. Poznan University of Medical Sciences, Chair and Department of Pharmaceutical Chemistry, Rokietnicka 3, 60-803 Poznań, Poland;
2. Doctoral School, Poznan University of Medical Sciences, 70 Bukowska, 60-812 Poznań, Poland;
3. Poznan University of Medical Sciences, Chair and Department of Chemical Technology of Drugs, Rokietnicka 3, 60-803 Poznań, Poland

jszkudlarek@ump.edu.pl

Keywords: *Psoriasis; 20-hydroxyecdysone; niosomes; nanoformulations*

Psoriasis is a chronic immune-mediated inflammatory skin disease characterized by excessive keratinocyte proliferation, infiltration of inflammatory cells, and dysregulated cell death. Topical therapy remains essential because it delivers drugs locally while minimizing systemic side effects [1,2]. Niosomes, non-ionic surfactant-based vesicular systems that mimic the bilayer structure of biological membranes, have gained increasing interest as carriers capable of enhancing dermal penetration and improving the topical delivery of bioactive compounds [3]. In this study, 20-hydroxyecdysone (20HE)-loaded niosomes were developed as a potential platform for the topical treatment of psoriasis. The formulations were prepared using the thin-film hydration method. Briefly, 20HE, surfactants, and cholesterol were dissolved in methanol, and the solvent was removed under reduced pressure via rotary evaporation to create a dry lipid film. The film was then hydrated with two selected media. Vesicle size was further reduced by sonication using a homogenizer. The impact of the hydration medium on formulation properties was evaluated. The optimized formulation was further characterized by NTA, DLS, ELS, HPLC, and pH measurement. Short-term stability was monitored over three weeks. The developed niosomal systems exhibited favorable physicochemical properties and maintained good stability during storage. Their pH was appropriate for potential topical application. The formulation composition and the choice of hydration medium influenced the final characteristics, with one medium showing significantly better performance than the other. Overall, these findings support further investigation of 20HE-loaded niosomes as a promising topical delivery system for psoriasis.

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Development of fluorinated triazinoindoles as highly potent and markedly selective aldose reductase inhibitors focused on prevention of diabetic complications

Terézia Teplanová[1], Ambróz Almássy[1], Andrej Boháč[1], Lucia Kováčiková[1,2]

1. Comenius University Bratislava, Faculty of Natural Sciences, Department of Organic Chemistry, Ilkovičova 6, 841 04 Bratislava, Slovak Republic
2. Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology, CEM v.v.i., Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic

teplanova16@uniba.sk

Keywords: *diabetes mellitus; diabetic complications; aldose reductase inhibitors; antioxidants; fluorinated oxotriazinoindoles*

A hyperglycemic state increases activation of the polyol pathway, which plays a key role in the pathogenesis of secondary diabetic complications. Inhibition of the first enzyme in the polyol pathway, aldose reductase, using therapeutics such as aldose reductase inhibitors (ARIs) has proven to be a promising target for the treatment of diabetic complications. In our previous projects, the ALR2 inhibitor cemtirestat (CMTI) was designed, which showed good inhibitory activity (IC_{50} = 116 nM), selectivity (S_F = 302) and also antioxidant properties. [1] The latter developed bioisoster OTI was much more effective (IC_{50} = 42 nM) and selective (S_F > 2 381) than CMTI. (Figure 1) [2] In order to further improve the inhibitor properties, we focused on the synthesis of a series of *in silico* designed fluorinated analogues of CMTI and its oxygen bioisostere otirestat (OTI) to increase activity, selectivity and drug-like properties. All these derivatives showed significant improvements in both activity and selectivity compared to unsubstituted analogues. The inhibitor (8-F)OTI proved to be most effective at nM concentration, IC_{50} = 15 nM, with a high selectivity towards ALR2 / ALR1 (S_F > 6 667). Position 8 on the aromatic ring of the indole skeleton of the inhibitor was shown, by molecular modeling, to be the best within the framework of complementarity to the ALR2 enzyme. Simultaneously, introducing a substituent at this position minimizes stabilizing interactions in ALR1, thereby increasing selectivity.

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Accurate profiling of EVOO secoiridoids for EFSA health claim validation: comparing UHPLC-ESI-HRMS approach against official methods.

Vavalà Angela[1], Bonacci Sonia[1], Oliverio Manuela[1], Nardi Monica[1], Procopio Antonio[1]

1. Department of Health Science, University Magna Græcia of Catanzaro, 88100 Catanzaro, Italy

angela.vavala@studenti.unicz.it

Keywords: Secoiridoids, Extra Virgin Olive Oil (EVOO), High-Resolution Mass Spectrometry (HRMS), Analytical Validation, EFSA Health Claim.

Extra Virgin Olive Oil (EVOO) is the key lipid component of the Mediterranean Diet, widely recognized as a functional food. Its biological properties (anti-inflammatory, antioxidant, and antiproliferative) are approximately 90% attributable to its phenolic fraction, particularly secoiridoids. The European Food Safety Authority (EFSA) has approved a health claim associating EVOO polyphenols with the protection of blood lipids from oxidative stress, applicable exclusively to oils containing at least 5 mg of hydroxytyrosol and its derivatives per 20 g of product. However, the industrial and pharmaceutical application of this claim is severely limited by the inadequacy of officially proposed quantitative methods, which rely on acid hydrolysis or express total phenols in standard equivalent units, thus providing only approximate estimations. A further critical issue is the poor availability of commercial reference standards for these complex molecules and the rapid chemical variations they undergo during storage.

The main objective of this study is to apply our already developed and validated UHPLC-ESI-HRMS method [1] for the direct and precise quantification of individual secoiridoid derivatives.

To overcome the lack of commercial references, seven biologically active compounds (tyrosol, hydroxytyrosol, oleocanthal, oleacein, oleuropein aglycone, verbascoside, and oleuropein) were previously synthesized in our laboratory using green semi-synthetic methodologies from olive oil by-products. Following an optimized hydroalcoholic extraction protocol, this validated molecule-by-molecule approach was tested on seven high-quality EVOO samples of certified origin to evaluate their chemical stability over time and unambiguously certify their nutraceutical properties. Crucially, the results were directly compared with the official standard method, which measures phenolic content merely in terms of tyrosol and hydroxytyrosol equivalents.

The goal of this comparison is to demonstrate how our true and selective quantification of bioactive secoiridoids easily overcomes the approximations of traditional protocols. Ultimately, this approach serves as a robust, essential analytical tool for the valorization of olive oil products, offering a detailed picture for the accurate certification of their therapeutic properties and EFSA compliance in the nutraceutical sector.

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ATTENDANTS

Surname	Name	Affiliation	Email
Albuquerque	Bàrbara Patricia	University of Porto	up201804981@up.pt
Alcaro	Stefano	Magna Græcia University of Catanzaro	alcaro@unicz.it
Altomare	Cosimo Damiano	University of Bari Aldo Moro	cosimodamiano.altomare@uniba.it
Arrighi	Francesca	Sapienza University of Rome	francesca.arrighi@uniroma1.it
Asim	Ayesha	Medical University of Lublin	ayesha.asim4@gmail.com
Atzeni	Giulia	University of Cagliari	giulia.atzeni@unica.it
Bandowski	Gyde	University of Hamburg	gyde.bandowski@studium.uni-hamburg.de
Barraja	Paola	University of Palermo	paola.barraja@unipa.it
Birsa	Mihail Lucian	Alexandru Ioan Cuza University of Iasi	lbirsa@uaic.ro
Bivacqua	Roberta	Magna Græcia University of Catanzaro	roberta.bivacqua@studenti.unicz.it
Bolognesi	Maria-Laura	University of Bologna	marialaura.bolognesi@unibo.it
Borges	Fernanda	University of Porto	fborges@fc.up.pt
Brogi	Francesco	University of Perugia	francesco.brogi@dottorandi.unipg.it
Buttitta	Giorgio	Sapienza University of Rome	giorgio.buttitta@uniroma1.it
Cagide Fagin	Fernando	University of Porto	fernandocagide@yahoo.es
Cardenas	Vince Bart	Università della Svizzera italiana	cardev@usi.ch
Carvalho Lima	Inês	University of Porto	nicar.2001@gmail.com
Castelôa	Mariana	University of Porto	up201606405@fc.up.pt
Cinque	Pierfrancesco	University of Naples Federico II	pierfrancesco.cinque@unina.it
Coluccia	Michele	Sapienza University of Rome	michele.coluccia@uniroma1.it
Corona	Angela	University of Cagliari	angela.corona@unica.it
Costi	Maria Paola	University of Modena and Reggio Emilia	mariapaola.costi@unimore.it
Cottiglia	Filippo	University of Cagliari	Filippo.cottiglia@unica.it
Dal Ponte	Nicolò	University of Padova	nicolo.dalponte@studenti.unipd.it
Damiani	Sara	University of Ljubljana	sara.damiani@ffa.uni-lj.si
Demuru	Laura	University of Cagliari	laura.demuru2@unica.it
Deplano	Alberto	University of Cagliari	alberto.deplano@unica.it
Deruvo	Caterina	University of Bari Aldo Moro	caterina.deruvo@uniba.it
Di Ricco	Fabio	University of Pisa	fabio.diricco@phd.unipi.it
Distinto	Simona	University of Cagliari	s.distinto@unica.it
Duran Martinez	Marta	University of Alcalá	m.duranm@uah.es
El Hadri	Chaimae	Ghent University	chaimae.elhadri@ugent.be
Fabbrizi	Emanuele	Sapienza University of Rome	emanuele.fabbrizi@uniroma1.it
Faloci	Federico	University of Padova	federico.faloci@phd.unipd.it
Ferrari	Silvia	MedChemExpress LLC	silvia.ferrari@medchemexpress.com
Ferro	Elsi	RS4 - Center for Research	elsi@crs4.it
Fois	Marzia	University of Ljubljana	marzia.fois@ffa.uni-lj.si
Fossati	Alice	University of Pavia	alice.fossati01@universitadipavia.it
Gandola Toledo	Maximiliano Javier	University of Cagliari	maximiliano.gandolatoledo@ugent.be
Garbagnoli	Martina	University of Pavia	martina.garbagnoli01@universitadipavia.it

Gargano	Adriana	Magna Græcia University of Catanzaro	adriana.gargano@studenti.unicz.it
Geronikaki	Athina	Aristotle University of Thessaloniki	geronik@pharm.auth.gr
Giuffrida	Stefano	University of Palermo	stefano.giuffrida@unipa.it
Greco	Ilario	Magna Græcia University of Catanzaro	grecoilario@gmail.com
Griffen	Ed	MedChemica Ltd	ed.griffen@medchemica.com
Gualtieri	Alessandro	Magna Græcia University of Catanzaro	alessandro.gualtieri002@studenti.unicz.it
Guglielmi	Paolo	Sapienza University of Rome	paolo.guglielmi@uniroma1.it
Jastrzebski	Michal	University of Lublin	michal.jastrz1998@gmail.com
Juza	Radomir	National Institute of Mental Health, Czech Republic	radomir.juza@nudz.cz
Kikely	Danijel	University of Ljubljana	Danijel.Kikelj@ffa.uni-lj.si
Kleszcz	Robert	Poznan University	kleszcz@ump.edu.pl
Konecny	Jan	University Hospital Hradec Králové	konecny77@seznam.cz
Kováčiková	Lucia	Slovak Academy of Sciences	lucia.kovacikova@savba.sk
Krajka-Kuźniak	Violetta	Poznan University	vkrajka@ump.edu.pl
Kratky	Martin	Charles University	martin.kratky@faf.cuni.cz
Kucerova	Marta	Charles University	kucerom@faf.cuni.cz
La Gatta	Valeria	University of Modena	valeria.lagatta@unimore.it
Lachowicz	Joanna Izabela	University of Cagliari	joanna.lachowicz@umw.edu.pl
Latajka	Rafal	Wroclaw University of Science and Technology	rafal.latajka@pwr.edu.pl
Laus	Antonio	RS4 - Center for Research	laus@crs4.it
Lavagna	Silvio Massimo	Sapienza University of Rome	silvio.lavagna@fondazione.uniroma1.it
Lemos	Leonardo	University of Santiago de Compostela	leonardo.censi@rai.usc.es
Lima	Carla	University of Porto	up201705563@up.pt
Litwin	Karolina		litwin.karrollinaa@gmail.com
Liviabella	Diletta	University of Perugia	diletta.liviabella@dottorandi.unipg.it
Lo Mascolo	Fabiana	University of Palermo	fabiana.lomascolo@unipa.it
Loche	Enrico	University of Cagliari	e.loche5@studenti.unica.it
Lupia	Antonio	University of Cagliari	antonio.lupia@unica.it
Maccioni	Elias	University of Cagliari	elias.maccioni@unica.it
Machado	Claudia Sofia	University of Porto	6704.claudia.sampaio@gmail.com
Majchrzak-Celińska	Aleksandra	Poznan University	majchrzakcelinska@ump.edu.pl
Mancini	Andrea	Sapienza University of Rome	an.mancini@uniroma1.it
Marchese	Emanuela	Magna Græcia University of Catanzaro	emanuela.marchese@live.it
Marquez-Cantudo	Laura	University San Pablo CEU	laura.marquezcantudo@ceu.es
Martinez	Marta Duran	University of Alcalá	m.duranm@uah.es
Martinez	Ana	CIB-CSIC	ana.martinez@csic.es
Matos	Maria	University of Santiago de Compostela	mariajoao.correiapinto@usc.es
Matosiuk	Dariusz	Medical University of Lublin	dariusz.matosiuk@umlub.edu.pl
Mazzochi	Ana Carolina	University of Santiago de Compostela	caromazzochi@gmail.com
Meier	Chris	University of Hamburg	chris.meier@uni-hamburg.de
Meleddu	Rita	University of Cagliari	rita.meleddu@unica.it

Mezeiová	Eva	University Hospital Hradec Králové	eva.mezeiova@fnhk.cz
Milli	Giorgio	University of Pavia	giorgio.milli01@universitadipavia.it
Moi	Davide	University of Cagliari	davide.moi@unica.it
Montalbano	Alessandra	University of Palermo	alessandra.montalbano@unipa.it
Moreira	Joana Filipa	University of Porto	joanamoreira-1997@hotmail.com
Musielak	Ewelina	Poznan University	emusielak@ump.edu.pl
Needle	Adam Anthony	Charles University	needlea@faf.cuni.cz
Novás	Manuel	University of Santiago de Compostela	manuel.novas@usc.es
Nunes	Bárbara	University of Porto	up201804372@edu.fe.up.pt
Onali	Alessia	University of Cagliari	alessia.onali@unica.it
Onnis	Valentina	University of Cagliari	valentina.onnis@unica.it
Pagliaro	Angela	Italian Institute of Technology (IIT)	angela.pagliaro2@unibo.it
Paradiso	Emiliano	University of Ljubljana	emiliano.paradiso@ffa.uni-lj.si
Paravati	Maria Rosaria	Magna Græcia University of Catanzaro	mrparavati@unicz.it
Piga	Martina	University of Ljubljana	martinapiga96@gmail.com
Pinto	Miguel	University of Porto	miguel.pinto@med.up.pt
Piwowarczyk	Ludwika	Poznan University	lpiwowarczyk@ump.edu.pl
Prilop	Lara	University of Hamburg	lara.prilop@uni-hamburg.de
Raffellini	Lorenzo	University of Pisa	lorenzo.raffellini@phd.unipi.it
Rapposelli	Simona	University of Pisa	simona.rapposelli@unipi.it
Rizzi	Stefano	MedChemExpress LLC	stefano.rizzi@medchemexpress.com
Rocha	Teresa	University of Porto	teresa.rocha1202@gmail.com
Roleira	Fernanda	University of Coimbra	froleira@ff.uc.pt
Salaj	Nebojša	MDPI	salaj@mdpi.com
Salvi	Sara	University of Pavia	salvi.sara2000@gmail.com
Salvucci	Benedetta	Istituto Superiore di Sanità	benedetta.salvucci@uniroma1.it
Sanna	Erica	University of Cagliari	erica.sanna@unica.it
Santo	Angela	University of Bari Aldo Moro	angela.santo@uniba.it
Sarbu	Laura Gabriela	Alexandru Ioan Cuza University of Iasi	laura.sarbu@uaic.ro
Šárová	Denisa	Charles University	sarovad@faf.cuni.cz
Scarpelli	Rosa	Magna Graecia University of Catanzaro	rosa.scarpelli@studenti.unicz.it
Secci	Daniela	Sapienza University of Rome	daniela.secci@uniroma1.it
Sequeira	Lisa	University of Porto	lsequeira@med.up.pt
Serra	Elisa	University of Cagliari	e.serra75@studenti.unica.it
Sissi	Claudia	University of Padova	claudia.sissi@unipd.it
Sousa Janela	João	University of Coimbra	jlsjanela@hotmail.com
Susino	Manuel	SepaChrom	manuel.susino@sepachrom.com
Svobodova	Barbora	University Hospital Hradec Králové	barbora.svobodova@fnhk.cz
Szkudlarek	Jagoda	Poznan University	jszkudlarek@ump.edu.pl
Tabarrini	Oriana	University of Perugia	oriana.tabarrini@unipg.it
Tack	Ruben	Ghent University	ruben.tack@ugent.be
Tavares da Silva	Elisiário	University of Coimbra	etavares@ff.uc.pt
Teplanova	Terezia	Comenius University	terezia.teplanova@gmail.com
Tinaglia	Flavia	University of Cagliari	f.tinaglia@studenti.unica.it
Tuveri	Ivan	University of Cagliari	i.tuveri1@studenti.unica.it

Vaccari	Melania Elettra	University of Pavia	melaniaelettra.vaccari01@universitadipavia.it
Van Calenbergh	Serge	Ghent University	serge.vancalenbergh@ugent.be
Van de Velde	Ewout	Ghent University	ewout.vandevelde@ugent.be
Van Hauwermeiren	Anouk	Ghent University	anouk.vanhauwermeiren@ugent.be
Vavalà	Angela	Magna Graecia University of Catanzaro	angela.vavala@studenti.unicz.it
Vernile	Sofia	Sapienza University of Rome	sofia.vernile@uniroma1.it
Vinsova	Jarmila	Charles University	vinsova@faf.cuni.cz
Vistoli	Giulio	University of Milan	giulio.vistoli@unimi.it
Vitale	Oriana	University of Naples Federico II	oriana.vitale@unina.it
Yardimci	Elif	Ankara University	yardimcie@ankara.edu.tr

VENUE

Hotel Flamingo Resort is a 4-star hotel located directly by the sea with a private beach of white sand in Santa Margherita di Pula about 40 km from Cagliari, one of the best holiday destinations in south Sardinia.


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Welcome to
XV Paul Ehrlich Euro-PhD
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20th-22nd May, Flamingo Hotel
Santa Margherita di Pula, Cagliari, Italy
E. MACCIONI
F. BORGES

The Organizing Committee welcomes you to the *Gala Dinner* at the Wild Duck conference hall, located in an exclusive area of the Hotel Flamingo Resort.


CLOSING REMARKS

On behalf of the Scientific Committee and the Organizing Committee, I would like to thank all participants for their valuable contributions to the success of the XV Paul Ehrlich MedChem meeting.

This edition of the meeting was attended by 137 participants who shared the results of their scientific research, confirming once again the strength and vitality of the Paul Ehrlich MedChem Euro-PhD Network. The meeting has provided an inspiring environment for scientific discussion, collaboration, and professional growth, particularly for our PhD students and young researchers, who represent the future of medicinal chemistry.

Finally, I would like to express my sincere thanks to all members of the Organizing Committees who dedicated a lot of energy and time to allow the meeting to proceed smoothly.

I sincerely hope that the scientific exchanges and personal connections established during these days will continue to inspire new collaborations and innovative research in the years to come.

It has been a great pleasure to welcome all of you, and I look forward to meeting you again at the next Paul Ehrlich MedChem Meeting.

Prof. Elias Maccioni

Meeting Chairman and Scientific Coordinator