Società Chimica Italiana
Divisione di Chimica Farmaceutica

NMMC

# BOOK OF ABSTRACTS

# XXVII National Meeting on Medicinal Chemistry

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

7N 15P 6C 9F 14

14th Young Medicinal Chemists' Symposium
Nuove Prospettive in Chimica Farmaceutica

Palazzo Del Prete, Piazza Cesare Battisti
Università degli Studi di Bari Aldo Moro
September 11-14, 2022

# *WELCOME MESSAGE*

The Scientific and Organizing Committees are pleased to welcome you in Bari for attending the 27th National Meeting in Medicinal Chemistry (NMMC27), organized by the *Medicinal Chemistry Division of the Italian Chemical Society* (DCF-SCI) and sponsored by the *European Federation of Medicinal Chemistry and Chemical Biology* (EFMC). Bari is a beautiful seaside town, rich in churches, palaces, piazzas, theatres and restaurants, with a special charm throughout the year.

NMMC27, jointly with the 14th Young Medicinal Chemists Symposium "Nuove Prospettive in Chimica Farmaceutica" (NPCF14), will give you the opportunity to share views about the challenges around the corner, as well as medicinal chemistry hot topics. After two years of virtual meetings, the 2022 edition of the NMMC/NPCF symposium will be a presential event, although restricted to fully vaccinated individuals.

The meeting will cover advances in drug discovery in major therapeutic areas, including the treatment of bacterial and viral infections, with a particular focus on the emerging COVID-19 pandemics, but also neurodegenerative and cardiovascular diseases, rare diseases, and cancer. In five scientific sessions, artificial intelligence and machine learning techniques as applied to drug discovery, the so-called "new modalities in medicinal chemistry" and the most recent advances in sustainable, chemical and biophysical technologies will be also featured. More importantly, participants will have the chance to establish and strengthen collaborative networks, a key option for successful research.

The meeting starts on September 11th with the Opening Ceremony and continues at the Aula Aldo Moro (Palazzo Del Prete, Piazza Cesare Battisti) from 12 to 14 September 2022. The scientific program will include five plenary and ten keynote lectures, as well as numerous oral and poster presentations. We actively encourage the participation of young scientists through dedicated grants and reduced registration fees.

More than 250 delegates from academia and industry are expected. Upon application, more than forty grants for young people, covering full registration fees and suitable accommodations, have been provided by DCF-SCI and other Institutional Sponsors.

Welcome in Bari!

| Prof. Gianluca Sbardella | Prof. Maria Laura Bolognesi | Prof. Cosimo D. Altomare |
|---|---|---|
| *Scientific Committee Chair* | *President DCF-SCI* | *Local Organizing Committee Chair* |

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Francesco Leonetti (co-chair, University of Bari)
Carlo Franchini (University of Bari)
Marcello Leopoldo (University of Bari)
Orazio Nicolotti (University of Bari)

# Scientific secretariat

Marco Catto
Enza Lacivita
Giovanni Lentini
Department of Pharmacy – Pharmaceutical Sciences
University of Bari Aldo Moro

# EFMC

European Federation
for Medicinal Chemistry
and Chemical Biology

EFMC is an independent association founded in 1969, representing 30 societies from 26 European countries, and more than **9000 scientists**. It's main objective is to **advance the science of medicinal chemistry and chemical biology.**

## Upcoming Events

### EFMC-YSN MedChemBioOnline

Webinars mixing science, soft-skills training
& round table discussions
www.efmc.info/efmc-ysn-medchembioonline

### 17th EFMC Short Course on Medicinal Chemistry

Oegstgeest, The Netherlands | April 23-26, 2023

### EFMC-ASMC'25
### IX International Symposium on Advances in Synthetic and Medicinal Chemistry

Zagreb, Croatia | September 3-7, 2023

### EFMC-YMCS 2023
### 10th EFMC Young Medicinal Chemists' Symposium

Zagreb, Croatia | September 7-8, 2023

### EFMC-ISCB 2023
### International Symposium on Chemical Biology

Basel, Switzerland | November 16-18, 2023

## Awards

— The Nauta Pharmacochemistry Award for Medicinal Chemistry and Chemical Biology
— The "UCB-Ehrlich Award for Excellence in Medicinal Chemistry"
— Prous Institute - Overton and Meyer Award for New Technologies in Drug Discovery
Visit **www.efmc.info/awards** for more information

## Prizes

— EFMC Prizes for Young Medicinal Chemists in Industry & Academia
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## EFMC-YSN

### The Young Scientists Network

Building a strong network at an early stage in your career is crucial!
The aim of the EFMC-YSN is to **inspire, connect** and **provide opportunities** to medicinal chemists and chemical biologists in their Early Career.
Visit **www.efmc.info/ysn** for more information

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# SCIENTIFIC PROGRAM

| Sunday, September 11th, 2022 | |
|---|---|
| 14.30-17.00 | **REGISTRATION** |
| | **Aldo Moro Hall** |
| 17.00-17.45 | **OPENING CEREMONY**<br>**Stefano Bronzini** – Rector of the University of Bari Aldo Moro<br>**Antonio F. Uricchio** – President of ANVUR<br>**Francesco Leonetti** – Head of the Department of Pharmacy-Pharmaceutical Sciences<br>**Luigi D'Ambrosio Lettieri** – Vice-president of F.O.F.I.<br>**Regional Political Authorities**<br>**Gianluca Sbardella** – Chair of the Meeting<br>**Maria Laura Bolognesi** – President of the Medicinal Chemistry Division<br>**Cosimo D. Altomare** – Chair of the Local Organizing Committee |
| 17.45-18.15 | **Medicinal Chemistry Division of the Italian Chemical Society's Awards**<br>**Francesco Merlino**, *University of Naples Federico II, Italy*<br>**Laura Scalvini**, *University of Parma, Italy*<br>**Best Doctoral Thesis Awards**<br>Design and Synthesis of new PET radiotracers in drug discovery<br>**Marco Maspero**, *University of Milan, Italy*<br>Design and synthesis of (pro)electrophilic compounds for investigating the multifactorial nature of neurodegenerative diseases: focus on inflammation-driven events<br>**Filippo Basagni**, *University of Bologna, Italy* |
| 18.15-18.45 | **Musajo Medal of the Medicinal Chemistry Division of the Italian Chemical Society**<br>Recipient: **Gabriele Costantino**, *University of Parma, Italy* |
| | **Chair: Maria Laura Bolognesi –** President of the Medicinal Chemistry Division |
| 18.45-19.45 | **PL1:** Exploring molecular promiscuity through activity data analysis and explainable artificial intelligence<br>**Jürgen Bajorath**, *University of Bonn, Germany* |
| 20.00 | **WELCOME BUFFET** |

| Monday, September 12th, 2022 | | | |
|---|---|---|---|
| | **Aldo Moro Hall** | | |
| | Chair: **Cosimo D. Altomare** | | |
| **9.00-9.50** | **PL2**: Going with the flow - the use of continuous processing for synthesizing Active Pharmaceutical Ingredients<br>**C. Oliver Kappe,** *University of Graz, Austria* | | |
| | **Aldo Moro Hall** | | **Vincenzo Starace Hall** |
| | Chairs: **Vincenza Andrisano**, **Orazio Nicolotti** | | Chairs: **Violetta Cecchetti, Stefano Alcaro** |
| **10.00-10.30** | **KN1:** Computational approaches to the design of covalent drugs<br>**Marco Mor,** *University of Parma, Italy*<br>***ChemMedChem Lecture*** | | **KN2:** Unleashing the potential of Translocator Protein as a therapeutic and diagnostic target: a successful MedChem tale<br>**Sabrina Taliani**, *University of Pisa, Italy* |
| **10.30-10.50** | **OC1:** SQM-Score: Universal Quantum-Mechanical Scoring Function for Structure-based Drug Design<br>**Adam Pecina**, *Czech Academy of Sciences Prague, Czech Republic* | | **OC2:** Functionalized 6*H*-dibenzo[c,e]thiazine 5,5-dioxides are potent suppressors of the toxicity mediated by the cellular prion protein<br>**Giuseppe Manfroni,** *University of Perugia, Italy* |
| **10.50-11.00** | **FC1:** Machine learning applied to early prediction of drug metabolism<br>**Marta Lettieri**, *S-IN Soluzioni Informatiche srl, Vicenza, Italy* | | **FC2:** Extra virgin olive oil extracts enriched in secoiridoids induce an anti-inflammatory profile in PBMCs from obese children<br>**Stefania De Santis,** *University of Bari, Italy* |
| **11.00-11.20** | **COFFEE BREAK** | | |
| **11.20-11.40** | **OC3:** The 3D-QSAR.COM portal as tool to develop predictive ligand-based and structure-based models for SARS-CoV-2 main protease inhibitors<br>**Rino Ragno**, *Sapienza University of Rome, Italy* | | **OC6:** Discovery of orexant and anorexant agents with indazole scaffold endowed with peripheral antiedema activity<br>**Adriano Mollica**, *University of Chieti-Pescara G. D'Annunzio, Italy* |
| **11.40-12.00** | **OC4:** Development of a LC-MS platform for monoclonal antibody characterization to assist the production of a rituximab biosimilar from plants<br>**Francesca Rinaldi**, *University of Pavia, Italy* | | **OC7:** New class of potential antidiabesity agents targeting DPPIV and CAs enzymes<br>**Laura Fumagalli**, *University of Milan, Italy* |
| **12.00-12.20** | **OC5:** A proof-of-concept of the analgesic effect of non-psychotropic *Cannabis sativa l.* and its main components on peripheral neuropathy<br>**Federica Pellati**, *University of Modena and Reggio-Emilia, Italy* | | **OC8:** Towards the characterization of corrector ARN23765 mechanism of action via photo-affinity labeling (PAL) approach<br>**Francesco Saccoliti**, *Italian Institute of Technology, Genoa, Italy* |
| **12.30-14.00** | **LUNCH** | | |
| **14.00-15.30** | **POSTER SESSION & COMMERCIAL EXHIBITION** | | |

# Monday, September 12th, 2022

| | Aldo Moro Hall | | Vincenzo Starace Hall |
|---|---|---|---|
| | Chair: **Gabriele Costantino**, **Giovanni Lentini** | | Chair: **Maria Laura Bolognesi**, **Roberto Di Santo** |
| **15.30-16.00** | **KN3:** Synthetic lethality for next generation precision oncology<br>**Andrea Cavalli,** *University of Bologna, Italy* | | **KN4:** A nature inspired approach to develop covalent enzyme inhibitors with anti-infective and anticancer activity<br>**Paola Conti**, *University of Milan, Italy* |
| **16.00-16.20** | **OC9:** New nicotinamide mimic scaffold allowed nanomolar inhibition of human PARP enzymes<br>**Oriana Tabarrini**, *University of Perugia, Italy* | | **OC11:** Broad spectrum metallo β-lactamases inhibitors: new tools against clinically-relevant carbapenemases<br>**Loretta Lazzarato**, *University of Turin, Italy* |
| **16.20-16.40** | **OC10:** Spindlin-1 degraders: stairway to heaven (?)<br>**Monica Viviano,** *University of Salerno, Italy* | | **OC12:** Novel dipeptide nitriles as antitrypanosomal agents targeting rhodesain of *Trypanosoma brucei rhodesiense*: development and combination studies<br>**Roberta Ettari**, *University of Messina, Italy* |
| **16.40-17.00** | **COFFEE BREAK** | | |
| **17.00-17.20** | **OC13:** TRPM8 ion channel: new target in the treatment of castration-resistant prostate cancer (CRPC)<br>**Veronica Di Sarno,** *University of Salerno, Italy* | | **OC15:** The PADAM oxidation route for the synthesis of SARS-CoV-2 main protease inhibitors<br>**Sveva Pelliccia**, *University of Naples Federico II, Italy* |
| **17.20-17.40** | **OC14:** Challenge transability of "in vitro" to "in vivo": gene-expression biomarkers and fluorescent image-guided surgery probes identification for ovarian cancer<br>**Antonio Scilimati**, *University of Bari, Italy* | | **OC16:** Discovery of diketo acid derivatives targeting the SARS-CoV-2 NSP13 helicase<br>**Valentina Madia**, *Sapienza University of Rome, Italy* |
| **17.40-17.50** | **FC3:** Combining mass spectrometry and nuclear magnetic resonance for the study of ligand:G-quadruplex interaction<br>**Erika Oselladore**, *University of Brescia, Italy* | | **OC17:** An integrated medicinal chemistry workflow for the development of new peptides as SARS-CoV-2 MPro covalent inhibitors<br>**Simona Musella,** *University of Salerno, Italy* |
| **17.50-18.00** | **FC4:** Carbazole derivatives as multi-target agents in breast cancer treatment<br>**Jessica Ceramella**, *University of Calabria, Italy* | | |
| **18.00-19:00** | **NETWORKING** | | |
| | **Tavola rotonda**<br>***Malattia di Lafora: dalla ricerca di farmaci ai diritti dei pazienti***<br>***Per una stretta cooperazione tra ricerca e assistenza***<br>**G. d'Orsi, T. Bressanello, G. Annichiarico, A. Liantonio, G. Costantino, C. Altomare** | | **Workshop**<br>**Why was my paper rejected? Optimizing manuscripts for successful submission and publication**<br>**David Peralta**, Editor-in-Chief ChemMedChem, Wiley-VCH |
| **19:30** | **CONCERT OF CORUS HARMONIA – BASILICA DI S. NICOLA** | | |

# Tuesday, September 13th, 2022

| | | | |
|---|---|---|---|
| | **Aldo Moro Hall** | | |
| | Chair: **Nicola A. Colabufo** | | |
| **9.00-9.50** | **PL3**: Innovative strategies to target non-coding RNAs with synthetic ligands<br>**Maria Duca**, *Université Côte d'Azur Nice, France* | | |
| | **Aldo Moro Hall** | | **Vincenzo Starace Hall** |
| | Chairs: **Patrizia Diana**, **Francesco Leonetti** | | Chairs: **Enza Lacivita**, **Tiziano Bandiera** |
| **10.00-10.30** | **KN5:** Targeting dopamine D4 receptor as a thrilling challenge to explore new therapeutic opportunities<br>**Fabio Del Bello**, *University of Camerino, Italy* | | **KN6:** Identification of ARN21641, an orally available and CNS penetrant Acid Ceramidase inhibitor with target engagement in mouse models of Gaucher and Krabbe diseases<br>**Rita Scarpelli**, *Italian Institute of Technology, Genoa, Italy* |
| **10.30-10.50** | **OC18:** Development of novel enzyme inhibitors of the endocannabinoids' catabolism for the treatment of epilepsy and neuroinflammatory conditions<br>**Stefania Butini**, *University of Siena, Italy* | | **OC19:** Hijacking the folding process for targeted protein degradation<br>**Andrea Astolfi**, *University of Perugia, Italy* |
| **10.50-11.00** | **FC5:** Targeting the mycobactin biosynthesis pathway in *M. tuberculosis*: a step towards the improvement of the anti-virulence activity of MbtI inhibitors<br>**Matteo Mori**, *University of Milan, Italy* | | **FC6:** Development of hydrogen sulfide-releasing hybrids as novel multitarget drugs<br>**Angela Corvino**, *University of Naples Federico II, Italy* |
| **11.00-11.20** | **COFFEE BREAK** | | |
| **11.20-11.40** | **OC20**: The pivotal role of pyrrolidine ring as multitarget scaffold in neurodegenerative diseases<br>**Antonio Carrieri**, *University of Bari, Italy* | | **OC23:** Nucleic acid aptamers: potential therapeutic agents for cancer and neurodegenerative disorders<br>**Jussara Amato**, *University of Naples Federico II, Italy* |
| **11.40-12.00** | **OC21:** Pursuing the complexity of bipolar disorder: rational design and optimization of first-in-class D3R/GSK-3β modulators towards an in vivo proof of concept<br>**Rita M.C. Di Martino**, *Italian Institute of Technology, Genoa, Italy* | | **OC24:** Combining quantum mechanics and machine learning in the search of the bioactive conformation of drug-like compounds<br>**Antonio Viayna**, *University of Barcelona, Spain* |
| **12.00-12.20** | **OC22:** S.M.A.R.T. steroids: synthesis and structure-activity relationship study towards allosteric modulators of *N*-methyl-D-aspartate receptors<br>**Eva Kudova**, *Czech Academy of Sciences Prague, Czech Republic* | | **OC25:** Challenging bioisosteric switch in AChE-MAO B dual-targeting hit optimization<br>**Leonardo Pisani**, *University of Bari, Italy* |
| **12.30-14.00** | **LUNCH** | | |
| **14.00-15.30** | **POSTER SESSION & COMMERCIAL EXHIBITION** | | |

| Tuesday, September 13th, 2022 | | | |
|---|---|---|---|
| | **Aldo Moro Hall** | | **Vincenzo Starace Hall** |
| | Chair: **Giannamaria Annunziato**, **Laura Scalvini** | | Chair: **Isabella Romeo**, **Francesco Merlino** |
| **15.30-16.00** | **KN7:** From the catalytic mechanism to the enzyme substrate selectivity: a study on *N*-acylethanolamine acid amidase<br>**Laura Scalvini,** *University of Parma, Italy* | | **KN8:** From natural resource to preclinical candidate: our experience with the temporin-derived peptide antimicrobial agents<br>**Francesco Merlino**, *University of Naples Federico II, Italy* |
| **16.00-16.10** | **FC7:** Discovery of 2-(4-hydroxy-3,5-dimethylphenyl)-*N*-(pyridin-2-yl)-1*H*-benzo[*d*]imidazole-6-sulfonamide as BET inhibitor with selectivity for the first bromodomain<br>**Alessandra Cipriano**, *University of Salerno, Italy* | | **FC11:** Tetrahydropyran and cyclohexane linked novel bacterial topoisomerase inhibitors with improved balanced antibacterial activity and safety profile<br>**Maja Kokot**, *National Institute of Chemistry, Ljubljana, Slovenia* |
| **16.10-16.20** | **FC8:** First-in-class selective inhibitors of the histone acetyltransferase KAT8<br>**Francesco Fiorentino**, *Sapienza University of Rome, Italy* | | **FC12:** Miconazole-like scaffold is a promising lead for developing *Naegleria fowleri*-specific brain permeable CYP51 inhibitors<br>**Valeria Tudino**, *University of Rome Tor Vergata, Italy* |
| **16.20-16.30** | **FC9:** Design, synthesis, and biological evaluation of new hybrid MOR agonist/HDACi compounds: an innovative approach for persistent pain management<br>**Giuliana Costanzo**, *University of Catania, Italy* | | **FC13:** Structural modifications of triazine-based compounds for high-efficiency PDK inhibition<br>**Camilla Pecoraro**, *University of Palermo, Italy* |
| **16.30-16.40** | **FC10:** Visible-light photocatalytic activity of isocyanides: from the proof-of-concept to the synthetic application in Ugi-like chemistry<br>**Camilla Russo**, *University of Naples Federico II, Italy* | | **FC14:** In silico assisted discovery of dual 5-LOX/sHE inhibitors: in vitro characterization and in vivo anti-inflammatory properties<br>**Tania Ciaglia**, *University of Salerno, Italy* |
| **16.40-17.00** | **COFFEE BREAK** | | |
| **17.00-17.30** | **In memoriam of Prof. Vincenzo Tortorella (1932-2022)**<br>**Celebration of retired colleagues** | | |
| **17.30-19.30** | **DCF-SCI GENERAL MEETING (ASSEMBLEA DELLA DIVISIONE DI CHIMICA FARMACEUTICA)** | | |
| **20.30** | **SOCIAL DINNER AT RISTORANTE ZONNO (Lungomare di Bari)** | | |

# Wednesday September 14th, 2022

| | | | |
|---|---|---|---|
| | **Aldo Moro Hall** | | |
| | Chair: **Marcello Leopoldo** | | |
| **9.00-9.50** | **PL4**: Targeting chemokine receptor CCR2 - From insurmountable antagonists to affinity-based probes<br>**Laura Heitman**, *Leiden University, The Netherlands* | | |
| | **Aldo Moro Hall** | | **Vincenzo Starace Hall** |
| | Chairs: **Marco Catto**, **Marcello Leopoldo** | | Chairs: **Gianluca Sbardella**, **Cosimo D. Altomare** |
| **10.00-10.30** | **KN9:** Development and hands-on application of PyRMD: a new AI-powered virtual screening tool<br>**Sandro Cosconati,** *Luigi Vanvitelli University, Naples, Italy* | | **KN10:** The discovery of potent and selective agonists of human transient receptor potential Cation Channel Subfamily M member 5: from HTS to early hit validation<br>**Alessio Barilli**, *Aptuit, an Evotec Company, Verona, Italy* |
| **10.30-10.50** | **OC26:** exploring CCRL2 Chemerin binding using accelerated molecular dynamics<br>**Antonio Coluccia**, *Sapienza University of Rome, Italy* | | **OC28:** The first in vivo proof-of-concept for the efficacy of selective HDAC6 inhibition in cystic fibrosis: anti-inflammatory profile, effects on bacterial load, formulation and biodistribution studies<br>**Margherita Brindisi**, *University of Naples Federico II, Italy* |
| **10.50-11.10** | **OC27:** Functionalized ligands targeting G protein-coupled adenosine receptors<br>**Stephanie Federico,** *University of Trieste, Italy* | | **OC29:** New insights in the development of cannabinoid receptor subtype 2 (CB2R) ligands<br>**Marialessandra Contino,** *University of Bari, Italy* |
| **11.10-11.30** | **COFFEE BREAK** | | |
| **11.30-11.50** | **OC30:** Optimizing the choice of 3D query structures in ligand-based virtual screenings with PharmScreen®<br>**Giorgia Zaetta,** *Parc Científic de Barcelona, Spain* | | **OC32:** Novel cyclic uPA-derived decapeptides reduce in vivo lung dissemination and re-educate CAF phenotype by acting through integrin αvβ5<br>**Alfonso Carotenuto**, *University of Naples Federico II, Italy* |
| **11.50-12.10** | **OC31:** A computational grid-based analysis to map drug-like peptide binding pockets of peptide-protein interactions systems<br>**Daniela Trisciuzzi,** *University of Bari, Italy* | | **OC33:** Screening of amino-acid-anthraquinone click chemistry conjugates targeting human telomeric G-quadruplexes<br>**Giovanni Ribaudo,** *University of Brescia, Italy* |
| | **Aldo Moro Hall** | | |
| | Chair: **Gianluca Sbardella** | | |
| **12.10-13.10** | **PL5**: COVID-19 pandemic: what we learnt in antiviral drug discovery, successes, and failures<br>**Vincenzo Summa,** *University of Naples Federico II, Italy* | | |
| **13.10-13.30** | **CLOSING REMARKS and POSTER PRIZES** | | |
| **13.30** | **LUNCH** | | |

# OVERVIEW OF THE SCIENTIFIC CONTRIBUTIONS

*Giovanni Lentini, Enza Lacivita and Marco Catto*

Herein, we tried to draw a roughly sketched picture of the participation and communication contents distilling some general notes from the submitted abstracts (160) for oral and flash communications (47) and poster presentations (113). Following is a graphical examination aimed at furnishing a general overview at a glance.
Based on the presenting authors, five Italian regions did not contribute any communication (Figure 1) but it might be kept in mind that scholars from those regions may appear in the abstract authorship.

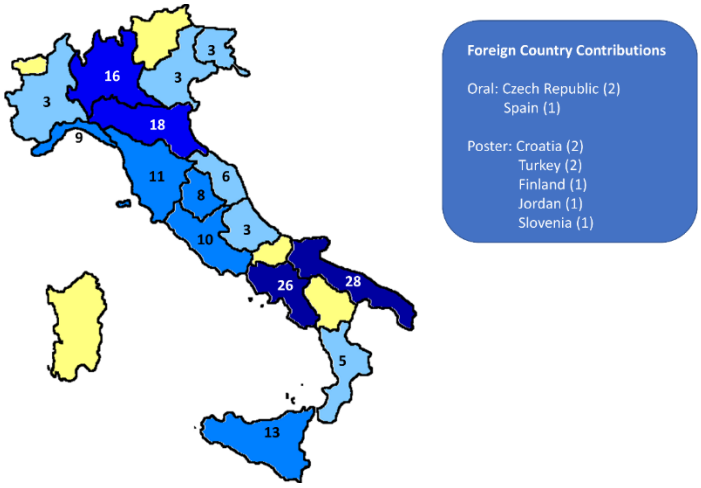


**Figure 1.** Contributors' provenance

The international feature of the Congress is somehow supported by the 10 foreign contributions and numerous (58) works performed in collaboration with foreign research centres (Figure 2).

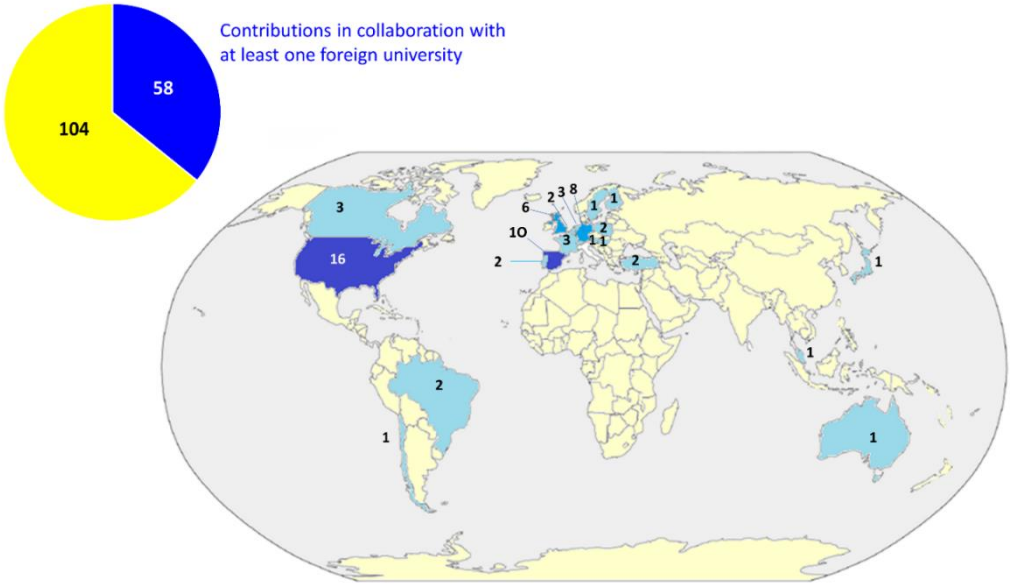


**Figure 2.** Foreign Universities involved in collaborative studies

The communication content uniformly overlaps the four main therapeutic areas with a relatively higher prevalence in the anti-cancer agent domain (Figure 3A). Among the autonomic function diseases, relevant is the position of the studies on cystic fibrosis. When antimicrobials are concerned (Figure 3B), the research on antifungals resulted in a relatively less explored domain. The antiprotozoal agents mainly targeted *P. falciparum* malaria and trypanosomiases.

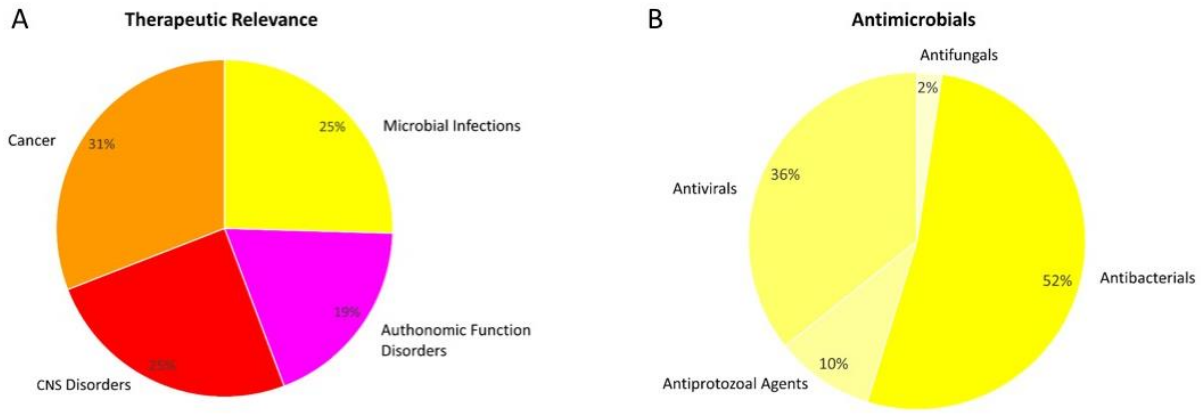


**Figure 3.** A, Communication content distribution per therapeutic area; B, Percentages of communications reporting on new antibacterials, antivirals, antiprotozoal agents, and antifungals.

Roughly, most of the communications reported on disorders related to excitotoxic damages in the CNS cells (neurones and glial cells). 'Other' includes epilepsy and bipolar disorder (Figure 4A). Most of the proposed works deal with enzyme inhibitors. The targets grouped under the 'Other proteins' tag include microorganism structural proteins and carrier proteins. Interesting is the prevalence of communications inspired by the multi-target strategy (Figure 4B).

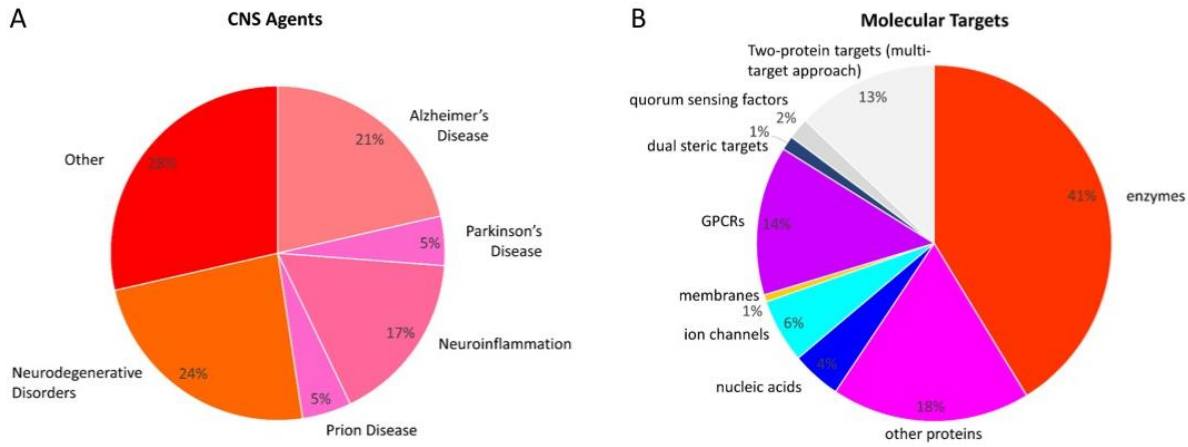


**Figure 4.** A, percentages of communications reporting on the various CNS disorders; B, communication distribution based on molecular targets.

As might be expected, the wide majority of communications presented new drugs based on heterocyclic systems (Figure 5A) with the largest group being the nitrogen-containing ones (Figure 5B). This trend is in perfect agreement with the prevalence of those systems in the yearly newly approved drugs. Someone suggested that this observation may be explained considering that enzyme inhibitors generally include heterocycles in their structures and that aliphatic heterocycles are generally introduced in the drug molecules as solubility enhancers.

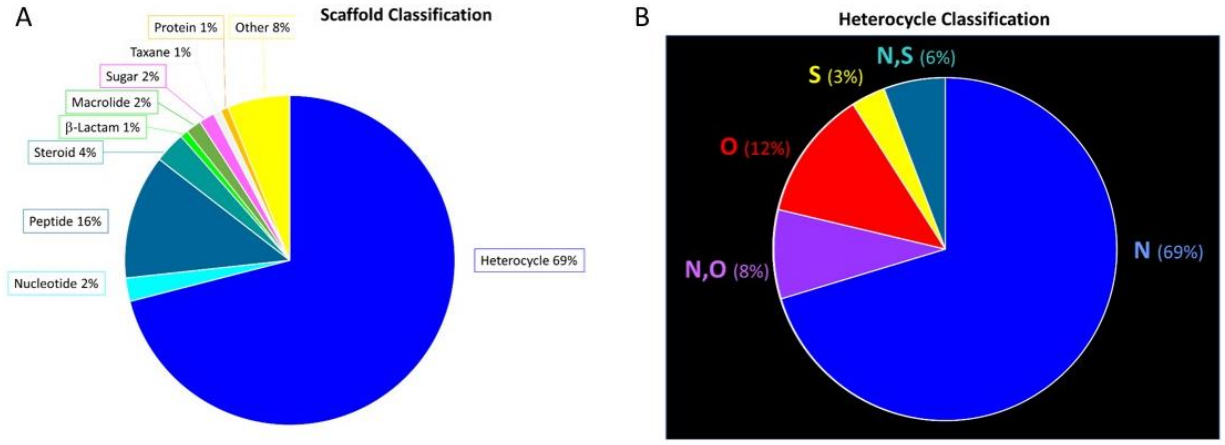


**Figure 5.** Communication distribution based on the type of scaffold (A) and of heterocycle (B) present in the structures of the reported new compounds.

# PLENARY LECTURES

PL-01

# EXPLORING MOLECULAR PROMISCUITY THROUGH ACTIVITY DATA ANALYSIS AND EXPLAINABLE ARTIFICIAL INTELLIGENCE

Bajorath, J.

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Multi-target activities of small molecules provide the basis of polypharmacology but are also responsible for undesired side effects in drug discovery. In addition, rationalizing multi-target activities is essential for drug repurposing. Furthermore, exploring the ability of small molecules to form "pseudo-specific" interactions with different targets helps to better understand molecular recognition phenomena and devise multi-target drug design strategies. Complementing experimental target profiling and proteomics, molecular promiscuity can be investigated in silico, for example, through systematic analysis of structural and activity data, "diagnostic" machine learning (ML) for hypothesis testing and explainable ML (XML). Computational approaches have been developed to rationalize promiscuity predictions and investigate structural features that distinguish multi- and single-target compounds. This methodological framework is generally applicable to identify chemical signatures of active compounds. Going forward, XML is expected to play an important role to further increase the acceptance of predictive modeling in the practice of medicinal chemistry.

PL-02

# GOING WITH THE FLOW – THE USE OF CONTNUOUS PROCESSING FOR SYNTHESIZING ACTIVE PHARMACEUTICAL INGREDIENTS

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Continuous flow processes form the basis of the petrochemical and bulk chemicals industry where strong competition, stringent environmental and safety regulations, and low profit margins drive the need for highly performing, cost effective, safe and atom efficient chemical operations. In contrast to the commodity chemical industry, however, the fine chemical industry primarily relies on its existing infrastructure of multipurpose batch or semi-batch reactors. Fine chemicals, such as drug substances and active pharmaceutical ingredients (APIs), are generally considerably more complex than commodity chemicals and usually require numerous, widely diverse reaction steps for their synthesis. These requirements generally make versatile and reconfigurable multipurpose batch reactors the technology of choice for their preparation. However, the advantages of continuous flow processing are increasingly being appreciated also by the pharmaceutical industry and, thus, a growing number of scientists, from research chemists in academia to process chemists and chemical engineers in pharmaceutical companies, are now starting to employ continuous flow technologies on a more routine basis.[1]

Flow technology has considerable advantages in mass- and heat transfer, safety and ease of scale-up, when compared to traditional batch reactions. Furthermore, hazardous chemistries such as highly exothermic reactions, or those involving unstable or toxic intermediates can be operated safely in flow, whereby this technology acts as a powerful route-enabler.

In this lecture, contributions from our research group in the field of continuous flow processing will be highlighted.[1-5] Emphasis will be given to highly atom efficient and process intensified chemical transformations useful for the synthesis of APIs or key intermediates that are often too hazardous to be executed in a batch reactor. These involve azide, diazomethane and nitration chemistry, oxidation reactions involving pure oxygen, catalytic hydrogenations and flow photochemistry/electrochemistry applications.

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PL-03

# INNOVATIVE STRATEGIES TO TARGET NON-CODING RNAS WITH SYNTHETIC LIGANDS

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RNA is one of the most intriguing and promising biological targets for the discovery of innovative drugs in a large number of pathologies and various biologically relevant RNAs that could serve as drug targets have already been identified.[1] Among the most important ones, it is worth to mention prokaryotic ribosomal RNA which is the target of a number of currently employed antibiotics, viral RNAs such as TAR, RRE and DIS RNA of HIV-1 or oncogenic microRNAs that are tightly involved in the development and progression of various cancers. However, difficulties in the rational design of strong and specific small-molecule ligands renders this kind of molecules relatively rare.
In this presentation, we will show our recent results about the structure-based design of new RNA ligands targeting oncogenic RNAs that led us to the identification of new compounds bearing a promising biological activity but also to a better understanding of the formed interactions toward the design of optimized compounds.[2] In parallel to the design of bioactive compounds, we also perform the screening of chemical library thus increasing the available chemical tools for the development of efficient and specific RNA binders for a wide number of therapeutic applications.[3] We will also show the validation of a new antibacterial target and the design of original compounds bearing potential antimicrobial activity against resistant bacterial strains.

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PL-04

# TARGETING CHEMOKINE RECEPTOR CCR2 - FROM INSURMOUNTABLE ANTAGONISTS TO AFFINITY-BASED PROBES

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In early drug discovery pharmaceutical companies optimize the properties of drug candidates for a given therapeutic target, focusing on standard pharmacological parameters of affinity, potency and intrinsic activity. Despite these intensive efforts, the success rate of a candidate drug moving to the pre-clinical development phase is disappointingly low.
We have recently focused on the chemokine receptor CCR2, which is a G protein-coupled receptor – an important family of drug targets. This receptor is primarily activated by the endogenous chemokine CCL2. Several small molecule antagonists have been developed to inhibit this receptor, as it is involved in many diseases characterized by chronic inflammation. Unfortunately, all these antagonists lack clinical efficacy warranting a better understanding of their mechanism of action.
During this talk, I will show how our work on novel drug discovery concepts (e.g. target binding kinetics and allosteric modulation) and novel tools, such as an AfBPP, contributes to this understanding, and how they open new avenues for CCR2, and GPCR small molecule drug discovery.

*PL-05*

# COVID-19 PANDEMIC: WHAT WE LEARNT IN ANTIVIRAL DRUG DISCOVERY, SUCCESSES AND FAILURES

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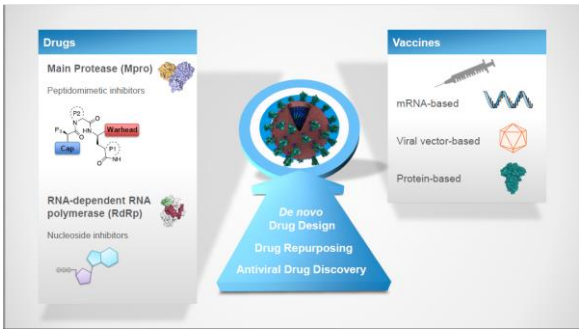
Covid-19 pandemic represents the most challenging and devastating worldwide infection of the last centuries. In this context, the scientific community with an unprecedented effort is deeply involved in the fight against SARS-CoV-2 infection approaching all possible strategies to find a solution to block the spread of the virus. To this purpose, innovative and traditional drug discovery strategies have been engaged, directed at both viral and host biological targets. While the global population was terrified by the pandemic and its consequence, the scientific community has produced very preliminary and hurried results - based more on the emotional stress than on a relevant scientific soundness - that were quickly demonstrated to be faulty or inconclusive. Nevertheless, the scientific rational approach cleaned up the initial chaotic scenario of possible treatments and was able to capitalize on all the previous experience acquired in antiviral drug discovery research. These focused efforts delivered, in less than three year, five vaccines and three drugs approved being a record for the drug discovery process. This lecture will focus on the small molecules approaches that led to the approval of three drugs and many candidates in clinical trial. In particular, the rapid evolution in the field will be showcased in parallel with the fast increasing knowledge of the virus and its pathophysiology. Target selection and drug development approaches will be presented, considering both failures and successful examples leading to the approved drugs. Finally, a perspective regarding the future development of the field and the so called 'preparedness strategy' will be also presented.

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# KEYNOTE LECTURES

KN-01

# COMPUTATIONAL APPROACHES TO THE DESIGN OF COVALENT DRUGS

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Covalent targeting of protein residues has emerged as a successful strategy to get effective drugs in different thearpeutic areas, including cancer, viral and other infectious diseases, sickle cell disease and platelet aggregation. A covalent drug can irreversibly inhibit an enzyme, overcoming competiton with high substrate concentration, or provide reversible interactions with the target, improving compound affinity. The design of a covalent drug must consider both compound recognition at the binding site and the propensity of the reactive warhead to selectively bind the target residue, avoiding or minimizing its intrinsic non-specific reactivity. While the arsenal of druggable warheads is gradually expanding beyond the classical acrylamide-based thiopiles, the ability to predict selective reativity toward a specific target is still a challenging task, in spite of the amount of structural information and of the improved computational techniques and resources now available.
Based on his experience in the field of endocannabinoid modulators and covalent kinase inhibitors, the speaker will present case studies on mechanistic modeling, by MD simulations and/or QM/MM hybrid simulations, of: active-site directed inhibitors of the 2-arachidonoylglycerol hydolyzing enzime, monoglyceride lipase;[1] reversible, covalent and allosteric inhibitors of the same enzyme;[2] the design of selective kinase inhibitors targeting the classical cysteine target of EGFR,[3] or the "catalytic" lysine by a sulfonyl fluoride warhed.[4] Exploring these cases, the influence of conformational equilibria, drug-target complementarity and warhead reactivity will be analyzed and discussed.

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KN-02

# UNLEASHING THE POTENTIAL OF TSPO AS A THERAPEUTIC AND DIAGNOSTIC TARGET: A SUCCESSFUL MEDCHEM TALE

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The Translocator Protein 18 kDa (TSPO), discovered in 1977 as an alternative binding site for the benzodiazepine diazepam, is an evolutionary well-conserved and tryptophan-rich 169-amino acids protein mainly stretching the outer mitochondrial membrane.[1] TSPO is implicated in a variety of fundamental cellular processes including steroidogenesis, heme biosynthesis, mitochondrial respiration, mitochondrial membrane potential, cell proliferation and differentiation, cell life/death balance, oxidative stress.[1] TSPO expression is ubiquitary, with higher levels in steroid producing tissues; in the central nervous system, it is mainly expressed in glial cells and in neurons. Altered TSPO expression has been found in some pathological conditions. In particular, high TSPO expression levels have been documented in cancer, neuroinflammation, and brain injury. Conversely, low TSPO expression levels have been evidenced in anxiety disorders. Therefore, TSPO is not only an interesting drug target for therapeutic purpose (anticonvulsant, anxiolytic, etc.), but also a valid diagnostic marker of related-diseases detectable by fluorescent or radiolabeled ligands.[2-5] Based on the research conducted in this field since 2004 in collaboration with national and international teams, the aim of this report is to present the most recent obtained results, in terms of both the identification of small-molecules with promising pharmacological profile and the development of fluoresecent- or radio-labelled probes with high potential as diagnostic tools, as well as the exciting future perspectives of targeting this multifaceted protein.

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KN-03

# SYNTHETIC LETHALITY FOR NEXT-GENERATION PRECISION ONCOLOGY

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Synthetic lethality is a mechanism that causes cell death when two genes are simultaneously compromised. In contrast, the cell survives when only either of the two genes is altered. Synthetic lethality was first demonstrated in Drosophila Melanogaster in 1922. Then, the concept has remained relatively unexploited until recently (after 2010), when scientists have looked at it in the pursuit of innovative anticancer compounds. In the last few years, the synthetic lethality paradigm has been extensively discussed within the oncology community, with the number of scientific contributions increasing exponentially.
Olaparib was the first PARP inhibitor (PARPi), followed by other PARPi drugs, used in BRCAness ovarian cancer. This drug family was then approved for breast and, since December 2019, for BRCAness pancreatic cancer. The connection between PARPi's and synthetic lethality was discussed and demonstrated retrospectively, with their discovery occurring somewhat serendipitously.
Our group has pioneered the field of synthetic lethality, developing a new concept, "small molecule-induced synthetic lethality," with the final objective of transforming a purely genetic mechanism into a chemical one. In this keynote lecture, we will present and discuss medicinal chemistry's role in discovering novel anticancer compounds that exploit the synthetic lethality mechanism. Starting with RAD51 and its role in the DNA double-strand break repair mechanism, we will present the most recent strategies/approaches in targeting mechanisms at the basis of the cancer genetic instability and how they can be turned into the Achille's heel of cancer cells. Subsequently, other backup pathways (including the repair mechanism, single-strand annealing) will also be illustrated, and some of its key molecular actors will be introduced. Interactions with clinicians will finally be discussed towards the practical application of synthetic lethality in precision oncology. We will conclude by illustrating the potential for evolution in the field through the combination of cancer immunotherapy and synthetic lethality, which has the potential to be one of the most challenging yet promising signs of progress in anticancer therapy in the coming years.

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Myers S.H. et al. J. Med. Chem. 2020, 63, 14151.
Patent Pending – Number 102019000023700, Dec. 11th 2019

# A NATURE INSPIRED APPROACH TO DEVELOP COVALENT ENZYME INHIBITORS WITH ANTI-INFECTIVE AND ANTICANCER ACTIVITY

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The last decade has witnessed a resurgence of interest in covalent inhibitors in medicinal chemistry, with several approved drugs, and many others under development.[1] Covalent inhibitors are composed of an *affinity element*, showing high affinity for the target protein, coupled to a *warhead*, which is an electrophilic group capable of forming a covalent bond with a nucleophilic amino acid residue. The covalent reaction should be driven by proximity and correct orientation of the warhead toward the specific residue. Thus, the control of warhead reactivity is a pivotal parameter in the development of covalent chemical tools and drug candidates. The ability to fine-tune warhead reactivity, avoiding off-target reactions with cellular nucleophiles including glutathione, could lead to high-quality irreversible chemical probes.

Nature is an excellent source of inspiration in the design of covalent inhibitors, since many natural products possess mildly electrophilic moieties able to covalently engage the target protein.[2]
Among the nature-derived irreversible molecules, acivicin (Fig. 1), produced by *Streptomyces sviceus,* has been reported to have significant anticancer, antimicrobial and antiparasitic activity.[3] Its biological profile is linked to the amino acidic structure, mimicking L-glutamine (*affinity element*), and to the 3-Cl-isoxazoline ring (*warhead*), which irreversibly reacts with the catalytic Cys of several target enzymes, mainly glutamine-dependent amidotransferases.[3] Through a direct comparison between acivicin and its synthetic 3-Br analogue (3-BA, Fig.1), we discovered that 3-BA was more potent than acivicin as *Trypanosoma brucei* CTP synthetase inhibitor, resulting in higher antiparasitic activity.[4]

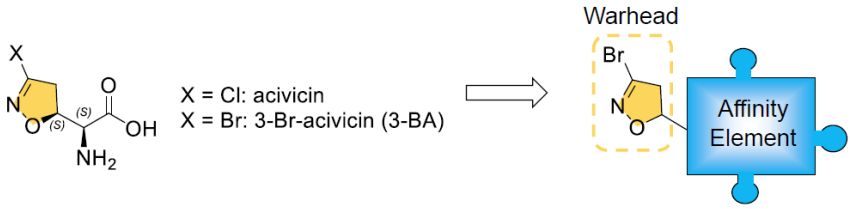


**Figure 1**

Afterwards, we exploited the 3-Br-isoxazoline scaffold as a novel *warhead* for the design of covalent inhibitors for different classes of cysteine-containing enzymes, by coupling it with selected *affinity elements*.
Herein, the development of covalent inhibitors of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) will be discussed. GAPDH is a key glycolytic enzyme that has raised considerable attention as a potential drug target in pathological conditions in which the glycolytic flux plays a crucial role, such as parasitic infections and cancer. Extensive investigation of the structure-activity relationships around the 3-bromo-isoxazoline core led us to identify promising antimalarial/antileishmanial agents showing high selectivity index with respect to mammalian cells.[5]

In addition, the results of our recent investigation of a panel of 3-bromo-isoxazoline derivatives as inhibitors of human recombinant GAPDH will be disclosed. Assessment of the antiproliferative effect on pancreatic ductal adenocarcinoma cells and in a mice xenograft model allowed to pinpoint one derivative as potential anticancer agent without apparent toxicity.

Interestingly, we also assessed the intrinsic reactivity of 3-Br-isoxazoline derivatives with thiols, demonstrating that this novel *warhead* has a moderate, drug-like reactivity, and could find broad application in the design of safe and effective covalent inhibitors.

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KN-05

# TARGETING DOPAMINE $D_4$ RECEPTOR AS A THRILLING CHALLENGE TO EXPLORE NEW THERAPEUTIC OPPORTUNITIES

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Since its identification and cloning in early 90s, the dopamine $D_4$ receptor ($D_4R$) has been suggested to be an attractive target for the treatment of neuropsychiatric diseases, such as schizofrenia. Unfortunately, $D_4R$ antagonists that were developed as antipsychotics failed in the clinic, making researchers lose their interest in $D_4R$ as a therapeutic target, and work in this area was silent for years.[1] Novel findings have renewed the interest in such a receptor as an emerging target for the management of different diseases, including brain cancers, alcohol or substance use disorders, cognitive deficits and eating disorders.[2] The resolved crystal structures of $D_4R$ in complex with the potent ligands nemonapride and L-745870 successfully helped to identify novel $D_4R$ chemotypes[3] and continue to support medicinal chemists in drug design. Further help in the design of new drugs may derive from the study of the $D_4R$ ligands showing functional selectivity for G-protein activation or β-arrestin recruitment, which will improve the knowledge of the biological functions associated with each pathway.

Herein, our recent advances on the development of subtype selective brain penetrant $D_4R$ ligands and their potential involvement in the treatment of some of the above-mentioned diseases will be presented. The new derivatives are analogs of the known $M_1$ muscarinic bitopic agonist 77-LH-28-1, that has recently demonstrated to be also a potent and subtype selective $D_4R$ antagonist (Figure 1).[4] Interestingly, 77-LH-28-1 bears an aliphatic butyl terminal, that makes it different from the classical piperidine/piperazine D4R agents reported so far.

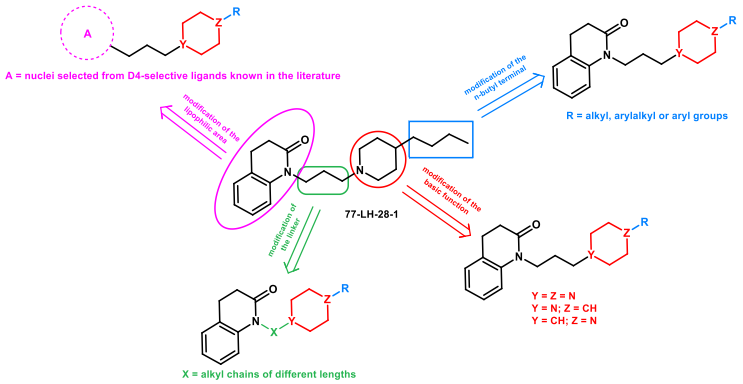


**Figure 1.** Modifications on the chemical structure of 77-LH-28-1

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KN-o6

# IDENTIFICATION OF ARN21641, AN ORALLY AVAILABLE AND CNS PENETRANT ACID CERAMIDASE INHIBITOR WITH TARGET ENGAGEMENT IN MOUSE MODELS OF GAUCHER AND KRABBE DISEASES

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Sphingolipids (SphLs) are a diverse class of bioactive molecules that are regulated by a complex network of enzymatic pathways.[1] A disturbance in these pathways leads to lipid accumulation and initiation of several SphL-related disorders. Acid ceramidase (AC) is a cysteine hydrolase that plays a crucial role in the metabolism of lysosomal ceramides and glycosphingolipids, which are important members of the SphL family.[2-3]

Herein, we present the lead optimization studies of a class of benzoxazolone (BOA) carboxamides (**Figure 1**). Targeted modifications on different positions of *Region A* and *B* of the scaffold led to the identification of the piperidine **22m**, as the first potent and selective human AC inhibitor (*h*ACi) with good oral bioavailability and brain penetration in mice. Furthermore, we demonstrated target engagement of **22m** in two animal models of neuropathic lysosomal storage diseases, Gaucher's and Krabbe's diseases (GD and KD).[4-6] After daily intraperitoneal administration at 90 mg/ kg, **22m** significantly reduced the brain levels of the toxic lipids glucosylsphingosine (GluSph) in 4L;C* mice (GD model) and galactosylsphingosine (GalSph) in Twitcher mice (KD model). These results suggest that **22m** is a lead molecule that can be further developed for the correction of neurological states of LSDs, where GluSph or GalSph plays a significant role in the disease pathogenesis.

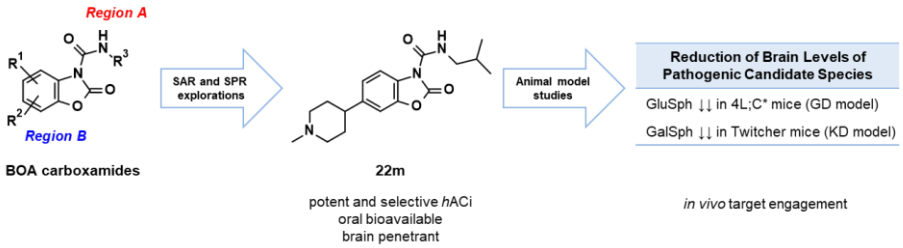


**Figure 1**

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KN-07

# FROM THE CATALYTIC MECHANISM TO THE ENZYME SUBSTRATE SELECTIVITY: A STUDY ON *N*-ACYLETHANOLAMINE ACID AMIDASE

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*N*-acylethanolamines (NAEs) are a large family of endogenous lipid transmitters characterised by an acyl chain, which differs among the NAEs for length and degree of unsaturation, linked to ethanolamine through an amidic bond. NAEs exert several biological effects, and among them palmitoylethanolamide (PEA) takes a major role in analgesia and in anti-inflammatory and neuroprotective processes mediated by the activation of PPARα[1]. The activity of PEA is terminated by its degradation to palmitic acid and ethanolamine catalysed by *N*-acylethanolamine acid amidase (NAAA), a member of the *N*-terminal nucleophile (Ntn) hydrolases superfamily, mainly expressed in B lymphocytes and macrophages,[2,3] where it concentrates in lysosomes and shows an optimal activity at acidic pH.[4] The catalytic mechanism of PEA hydrolysis has been recently investigated by free-energy studies in the QM/MM framework,[5] which have shown that acylation represents the critical step of the reaction, with the catalytic cysteine acting both as an acid, to protonate the ethanolamine leaving group, and as a nucleophile, to attack the PEA carbonyl carbon. The ethanol fragment of PEA did not appear to play an indispensable role in acylation, a result further supported by kinetic experiments showing that NAAA hydrolyses palmitoyl methyl amide (PMA) with high catalytic efficiency. On the other hand, a complete overview of the substrate selectivity of the enzyme is required, and while it is known that NAAA preferentially hydrolyses saturated fatty acid ethanolamides (FAEs), a detailed profile of the relationship between catalytic efficiency and fatty acid-chain length or modifications of the leaving group would be of critical relevance for the design of specific substrates.

In this report the effects of acyl chain or polar head modifications on substrate recognition and hydrolysis by NAAA have been investigated. The results of molecular modelling studies combined with biochemical studies reveal that the catalytic efficiency is strictly dependent upon fatty acyl chain length, whereas there is a wider tolerance for modifications of the polar head.[6] This relationship reflects the relative stability of enzyme-substrate complexes in molecular dynamics simulations.

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KN-o8

# FROM NATURAL RESOURCE TO PRECLINICAL CANDIDATE: OUR EXPERIENCE WITH THE TEMPORIN-DERIVED PEPTIDE ANTIMICROBIAL AGENTS

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Naturally occurring antimicrobial peptides (AMPs) are valid tools to tackle the antibiotic resistance phenomenon as they act thorough alternative mechanisms of actions with respect to conventional antibiotics.[1] Among them, the frog skin-derived Temporins possess interesting properties for biological investigations due to *i)* short sequence length, *ii)* activity against a wide range of pathogens, *iii)* additional chemotactic activity and immunomodulatory effects.[2] The 13-mer temporin L (TL) peptide is one prominent isoform, showing elevated antimicrobial potency, strong affinity for Gram-negative such as *P. aeruginosa* and *E. coli*, although it also exhibits a significant hemolytic activity at microbicidal concentrations.[3] Our recent efforts have been aimed at improving its therapeutic index and drug-like features through the application of different chemical approaches. A library of macrocyclic peptide analogues of TL was obtained by embracing different intramolecular linking strategies (*e.g.,* lactam, 1,4-triazolic and hydrocarbon bridges),[4] while chemical tag motifs, including fatty acids, were introduced at key locations, to further control target cell specificity.[5] From these studies peptidomimetics with greater broad-spectrum antimicrobial activity, including resistant bacterial strains, and less toxicity, were identified as encouraging pre-clinical candidates to be further assessed for the development of new antimicrobial "weapons" with biomedical applications.

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*KN-09*

# DEVELOPMENT AND HANDS-ON APPLICATION OF PYRMD: A NEWLY DEVELOPED AI-POWERED VS TOOL

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A new artificial intelligence (AI)-powered virtual screening (VS) software will be presented in this contribution. In particular, the Random Matrix Discriminant (RMD) algorithm has been implemented in a new fully automated tool called PyRMD.[1] This ligand-based VS tool is trained using target bioactivity data directly downloaded from the ChEMBL repository without manual intervention. The software automatically learns the distinctive chemical features responsible for the compounds' activity/inactivity. PyRMD allows to easily screen millions of compounds in hours through an automated workflow and intuitive input files allow to finely tune each parameter of the calculation. In addition to the description of this new tool, its application to the discovery of polypharmacological anticancer agents will be discussed along with the obtained biological effect of the identified leads. Finally, preliminary results will be described on the application of PyRMD in massive receptor-based VS calculations.

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KN-10

# THE DISCOVERY OF POTENT AND SELECTIVE AGONISTS OF HUMAN TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL SUBFAMILY M MEMBER 5: FROM HTS TO EARLY HIT VALIDATION

Barilli, A.;[a] Aldegheri, L.;[a] Bianchi, F.;[a] Castelletti, L.;[a] Lingard, I.;[a] Nola, S.;[a] Piccoli, L.;[a] Pompilio, D.;[a] Raveglia, L.;[a] Salvagno, C.;[a] Tassini, S.;[a] Virginio, C.;[a] Brault, L.;[a] Brodbeck, D.;[a] Feriani, A.;[a] Myers, R.;[b] Sabat, M.[b]

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Transient Receptor Potential cation channel subfamily M member 5 (TRPM5) is a non-selective monovalent cation channel activated by intracellular $Ca2^{+}$ increase. Within the gastrointestinal system, TRPM5 is expressed in the stoma, small intestine, and colon. In the search for novel chemical matter to be utilized as a starting point for the development of potent and selective agonists of hTRPM5, a High Throughput Screening approach was selected. A diversity-based compound library was screened with a fluorometric imaging plate reader (FLIPR) membrane potential (FMP) assay protocol. The first hits were progressed to hit confirmation and to two counter assays to exclude false positives, and the resulting confirmed hits were further selected for follow up with concentration-response curve based on several criteria: TRPM5 activity in the FMP assay higher than a defined threshold, low activity in a $Ca^{2+}$ flux assay and low activity on the CHO-wild type in an FMP assay. From CRC, the most promising compounds were selected to be profiled in the electrophysiology assay and clustered and prioritized based on potency and calculated properties. This process led to the discovery of 16 potentially interesting chemical series for which the Early Hit Validation was performed by means of compound acquisition and wet chemistry.[1]

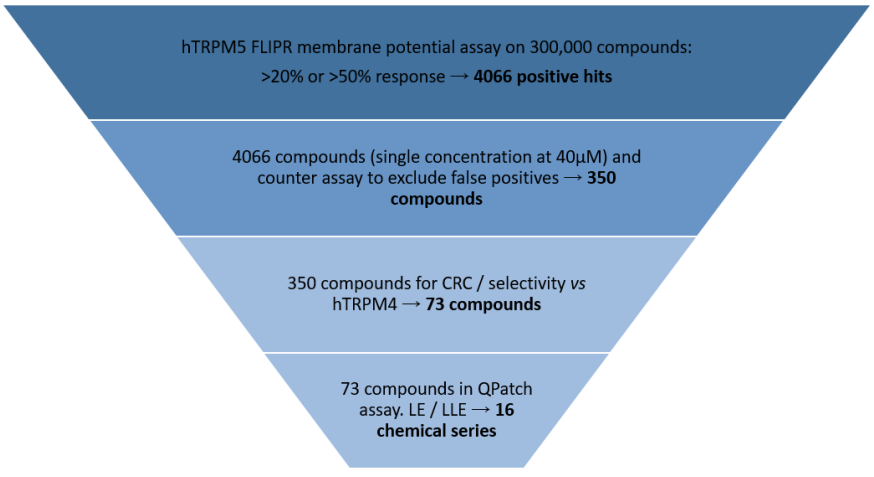


***Figure 1: HTS screening cascade***

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# ORAL COMMUNICATIONS

# SQM-SCORE: UNIVERSAL QUANTUM-MECHANICAL SCORING FUNCTION FOR STRUCTURE-BASED DRUG DESIGN

Pecina, A.;[a] Fanfrlik, J.;[a] Lepsik, M.;[a] Köprülüoglu, C.;[a] Eyrilmez, S.;[a] Rezac, J.[a] and Hobza, P.[a]

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Due to the enormous advances in computational power, quantum mechanical (QM) methods have been incorporated into the process of drug design in all its preclinical stages. Last decade, QM methods have been gaining in importance especially in structure-based drug design, where a reliable description of protein-ligand interactions is of utmost significance. However, strategies i.e. QM/MM, fragmentation or semiempirical (SQM) methods have to be pursued to overcome the unfavourable scaling of QM methods.

Parametrizations of SQM and implicit solvent methods in our laboratory have been instrumental to obtain a reliable SQM-based scoring function.[1-3] The experience gained in its application for activity ranking of ligands binding to tens of protein targets resulted in setting up a faster scoring approach, i.e. *SQM-Score,* which outperforms standard scoring methods in native pose identification for two dozen protein targets with ten thousand poses.[4-6] The *SQM-Score* was also tested in the first affinity ranking study that used a challenging set of 10 inhibitors binding to carbonic anhydrase II metalloprotein through Zn ion in the active site.[7] Recently, *SQM-Score* was effectively applied in a proof-of-concept study in virtual screening with the aim to prioritize 72 active ligands over ~4500 inactive ones toward a HSP90 target.[8]

Here, we present a large-scale affinity ranking performance of *SQM-Score* tested on a unique dataset of ten diverse protein systems with high-resolution crystal structures, each in a complex with >10 ligands with known experimental inhibitory constant ($K_i$) or $IC_{50}$. We are confident that the successful applications of *SQM-Score* and its feasibility and chemical generality showed its great potential to become a general tool during the hit-to-lead stage of structure-based drug design.[3]

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# FUNCTIONALIZED 6*H*-DIBENZO[*c*,*e*]THIAZINE 5,5-DIOXIDES ARE POTENT SUPPRESSORS OF THE TOXICITY MEDIATED BY THE CELLULAR PRION PROTEIN

Manfroni, G.;[a] Felicetti, T.;[a] Cernicchi, G.;[a] Barreca, M.L.;[a] Fallarino, F.;[b] Cecchetti, V.;[a] Biasini, E.[c]

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Prion diseases, usually rapidly progressive and always fatal, are rare neurodegenerative conditions of human and animals associated with conformational changes of the cellular prion protein ($PrP^C$) to the scrapie form ($PrP^{SC}$).[1] $PrP^C$ itself has been shown to participate in several other pathologies of the nervous system by acting as a toxicity-transducing receptor for different misfolded protein isoforms.[2,3] A growing body of evidences indicate that $PrP^C$ plays a dual role in prion diseases by supporting the $PrP^{Sc}$-templated propagation and by mediating itself neurotoxic effects, underscoring a distinction between prion infectivity $PrP^{Sc}$-mediated and $PrP^C$-induced toxicity.[4,5] Indeed, as an alternative way to the "classic" approach of inhibiting the $PrP^C$ into $PrP^{Sc}$ conversion, blocking the prion-mediated downstream neurotoxic signals is another possible strategy for treating prion diseases, especially those caused by a mutated $PrP^C$, such as the Creutzfeldt-Jakob disease and the Gerstmann-Straüssler-Scheinker syndrome. However, no current single assay can bring out the anti-prion activity of a compound acting on the $PrP^C$ to $PrP^{Sc}$ conversion and/or on the $PrP^C$-induced neurotoxicity. In this context, the Drug-Based Cellular Assay (DBCA), a novel cellular assay for studying mutant PrP-related toxicity, represents a unique tool to identify compounds capable of suppressing the toxicity induced by a mutated version of PrP (ΔCR).[6] Screenings of chemical libraries by using DBCA led to several promising hits, including the commercial 6*H*-dibenzo[*c*,*e*]thiazine 5,5-dioxide analogue (**LD24**),[7] that was selected by us as starting hit due to our chemical experience/expertise in managing this scaffold. In this work,[8] we report the medicinal chemistry optimization process performed around **LD24** carried out by combining the selection of commercially available analogues with the ligand-based design and synthesis of a large series of new derivatives. Medicinal chemistry efforts culminated with the identification of some dibenzothiazine compounds capable to rescue, at nM concentrations, cell viability reduced by ΔCR $PrP^C$ signal toxicity in HEK cells (DBCA). Several compounds showed a strong potency improvement with respect to **LD24**. In depth experiments displayed that a compound potently inhibited mutant PrP currents and abrogated Aβ-induced and $PrP^C$-dependent detrimental effects in primary neurons. Interestingly, one of the most potent compounds also exhibited the ability to rescue $PrP^{Sc}$-delivered synaptotoxicity in mouse brain slices. The mechanism of action for these compounds is still unknown as none of them was able to directly bind $PrP^C$ or inhibit prion replication in cell cultures or brain slices. Accordingly, we prepared a biotinylated probe to perform target fishing experiments which are currently underway.

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# THE 3D-QSAR.COM PORTAL AS TOOL TO DEVELOP PREDICTIVE LIGAND-BASED AND STRUCTURE-BASED MODELS FOR SARS-COV-2 MAIN PROTEASE INHIBITORS

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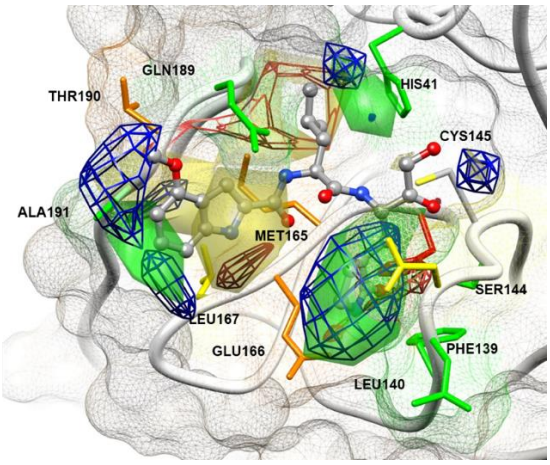
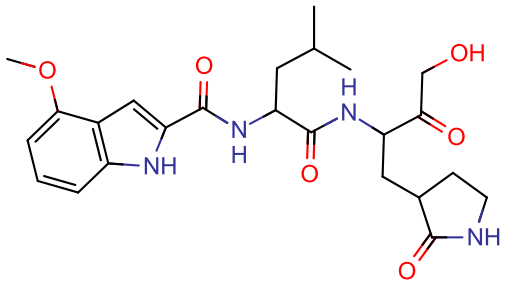
The SARS-Cov-2 main protease[1] (Mpro) of is the essential enzyme for maturation of functional proteins implicated in viral replication and transcription. The peculiarity of its specific cleavage site joint with its high degree of conservation among all coronaviruses promote it as an attractive target to develop broad-spectrum inhibitors, with high selectivity and tolerable safety profile. Herein is reported a combination of three-dimensional quantitative structure-activity relationships (3-D QSAR) and comparative molecular binding energy (COMBINE) analysis to build robust and predictive ligand-based (LB) and structure-based (SB) statistical models, respectively. Models were trained on experimental binding poses of co-crystallized Mpro-inhibitors and validated on available literature data. Analysis of the models led to a joint LB-SB unique 3-D structure-activity relationships (3-D SARs, Figure 1) that were found in agreement with nirmatrelvir chemical features properties, a novel oral Mpro-inhibitor that has recently received U.S. FDA emergency use authorization (EUA) for the oral treatment of mild-to-moderate COVID-19 infected patients.
Predictive ability of the models was assessed by means of crystallized and modeled test set.
As most of calculation were performed through the web portal 3d-qsar.com[2] the results confirmed the portal as a useful tool for drug design




**3**

**Figure 1**. Graphical depiction of 3-D QSAR plots in the binding site of compound **3** (gray) - $M^{pro}$ minimized complex (PDB code = 6XHM). Residues are colored depending on their higher activity contribution: green - STE positive, yellow - STE negative, red - HB positive, orange - DRY positive (see legend in Figure 5).

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OC-04

# DEVELOPMENT OF A LC-MS PLATFORM FOR MONOCLONAL ANTIBODY CHARACTERIZATION TO ASSIST THE PRODUCTION OF A RITUXIMAB BIOSIMILAR FROM PLANTS

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Despite the high efficacy and safety of mAbs, the high costs associated with their traditional production process in mammalian host cell lines still represent a serious limit.[1] The use of plants as expression systems to produce functionally active recombinant therapeutic proteins started 1989 and has significant potential advantages such as easy scalability and distribution and low production costs.[2,3] However, the production of mAbs in different expression systems might result in species-specific modifications which reflect the unique features of the biochemical machinery typical for each production environment, which can negatively impact on protein structure and properties. In this context, a detailed structural characterization of the product is a crucial aspect that can address the selection of the most appropriate expression system or support the choice of engineering strategies to obtain the desired product.

Rituximab (RTX) is the first monoclonal antibody (mAb) approved for clinical use in the therapy of cancer. It is a mouse/human chimeric monoclonal antibody produced in Chinese hamster ovary (CHO) mammalian cells and directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. RTX has been approved for the treatment of various lymphoid malignancies, including B-cell non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia, and more recently its therapeutic indication has been extended to other areas such as autoimmune disorders. The patent on the original Rituximab products, MabThera (EU) and Rituxan (US), expired in 2013 and 2016, respectively.[4] Thus, the development of RTX biosimilars is an interesting goal for biotech companies.

In this presentation, an analytical platform based on the use of liquid chromatography (LC) and mass spectrometry (MS) will be discussed as a tool for the characterization of RTX obtained from two plant expression systems (rice and tobacco) in comparison to the mammalian cell-derived reference mAb.

Reversed phase liquid chromatography (RPLC), hydrophilic interaction liquid chromatographic (HILIC), cation exchange chromatography (CEX) and size exclusion chromatography (SEC) methods, hyphenated to UV or high resolution MS detection, were developed. The application of the methods to intact or digested mAbs allowed to assess several critical attributes such as primary structure, glycan composition, species-related heterogeneity, glycosylation degree, charge variant, aggregation tendency and enzymatic stability.

Data highlighted the features and criticalities of each production approach; the use of rice led to heterogeneous but stable products over time, suggesting the absence of proteases in seeds, while tobacco expression system generated mAbs with a more homogeneous glycosylation profile but characterized by a lower stability probably due to the presence of proteases.

The developed analytical system supports the selection of the best plant expression system and guides the choice of engineering strategies for the production of recombinant humanized mAbs of interest.

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# A PROOF-OF-CONCEPT OF THE ANALGESIC EFFECT OF NON-PSYCHOTROPIC *CANNABIS SATIVA* L. AND ITS MAIN COMPONENTS ON PERIPHERAL NEUROPATHY

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Neuropathic pain affects 7–10% of the world population.[1] Multiple causes of neuropathic pain have been described and its incidence is likely to increase owing to the ageing global population, increased incidence of diabetes mellitus and improved survival from cancer after chemotherapy. Current therapies include antidepressants, antiepileptics, local anesthetics and opioids. [1] These treatments are effective in less than 50% of patients, and they are characterized by many side effects, that limit their long-term use. [1]
*Cannabis sativa* L. is used in the treatment of chronic pain, thanks to its interaction with the endocannabinoid system. The most investigated cannabinoids are $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and cannabidiol (CBD), but the psychotropic properties of $\Delta^9$-THC, involving its interaction with CB1 receptors, affect patient quality of life, thus making cannabis unsuitable for a long-term medical use.[2]
For these reasons, in this work we evaluated the possible anti-hyperalgesic effect of non-psychotropic *C. sativa* oil extracts in animal models of acute and persistent pain. To identify the possible synergy and interaction between cannabinoids and terpenes, we compared an extract devoid of terpenes and with high a concentration of CBD (K1) with an extract containing a high level of both cannabinoids and terpenes (K2).
The detailed chemical characterization the oil extracts was carried out by ultra-high-performance liquid chromatography (UHPLC), coupled with high-resolution mass spectrometry (HRMS), using a target metabolomic approach. Among all the identified cannabinoids, the most representative ones were further quantified by HPLC-UV.[3] Volatile compounds in the oils were fully analyzed by GC-MS.[3]
The anti-hyperalgesic effect of K2 and K1 after oral administration was assessed by measuring mechanical and thermal allodynia in spared nerve injury (SNI) mice. The mechanism of action of K2 was investigated using spinal cords samples in a cell-based *in vitro* model of neuroinflammation. Oral administration of K2 at 25 mg $kg^{-1}$ ameliorated mechanical and thermal allodynia in SNI mice, with a rapid and a long-lasting effect, without inducing an alteration of locomotor activity, with an efficacy higher than K1. K2 reduced p-ERK, p-p38 and p-JNK1 protein levels compared to the untreated mice. Moreover, the extract downregulated protein expression of neuroinflammatory factors (HDAC-1, p-65, NOS2).
To deeper investigate the possible synergism of CBD and β-caryophyllene (BCP), which are the two main compounds among cannabinoids and terpenes, respectively, an *in silico* structure-based approach was performed on CB2 receptors. CBD and BCP were simultaneously docked into the binding site of CB2 (PDBID: 5zty), using AutoDock Vina 1.2.0. CBD was predicted to bind close to the binding site entrance, while BCP was docked deeper into the binding site. Together, CBD and BCP were able to resemble the experimental binding mode of the reference ligand and the interactions with the binding site residues better than CBD or BCP alone, which could explain the higher activity of K2 compared to K1. *In vitro* binding and functional assays are currently ongoing to validate the *in silico* results.

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# DISCOVERY OF OREXANT AND ANOREXANT AGENTS WITH INDAZOLE SCAFFOLD ENDOWED WITH PERIPHERAL ANTIEDEMA ACTIVITY

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The endocannabinoid system represents an integrated neuronal network involved in the control of several organisms' functions, such as feeding behavior. A series of hybrids of 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (rimonabant), a well-known inverse agonist of the type-1 cannabinoid receptor (CB1), once used as an antiobesity drug, and the N-(2S)-substitutes of 1-[(4-fluorophenyl)methyl]indazole-3-carboxamide with 1-amino-3-methyl-1-oxobutane (AB-Fubinaca), 1-amino-3,3-dimethyl-1-oxobutane (ADB-Fubinaca), and 3-methylbutanoate (AMB-Fubinaca), endowed with potent agonistic activity towards cannabinoid receptors CB1 and CB2 were synthetized in solution as C-terminal amides, acids, methyl esters and N-methyl amides. These compounds have been studied by binding assays to cannabinoid receptors and by functional receptor assays, using rat brain membranes in vitro. The most active among them as an agonist, (S)-1-(2,4-dichlorobenzyl)-N-(3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-1Hindazole-3-carboxamide (LONI11), and an antagonist, (S)-2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)-3-methylbutanoic acid (LONI4), were tested in vivo in mice, to evaluate their ability to stimulate or suppress feeding behavior after intraperitoneal (i.p.) administration.

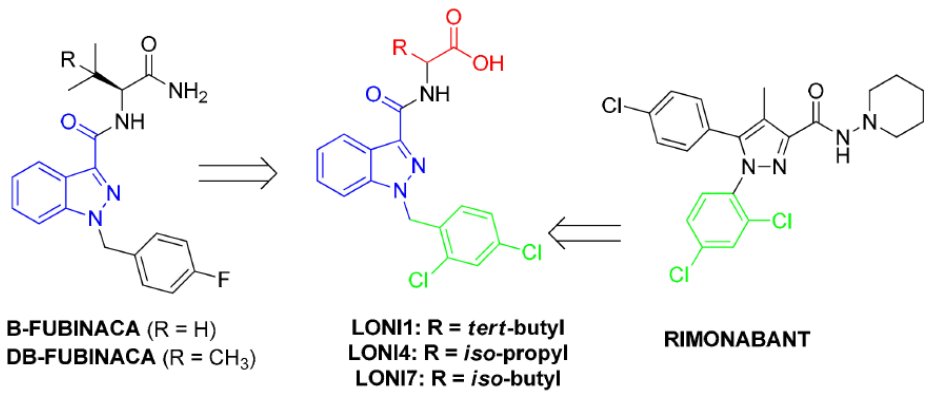


**Figure 1**

For a LONI11 formalin test and a tail flick test after an administration by the subcutaneous (s.c.) and intracerebroventricular (i.c.v.) routes, respectively, were also carried out in vivo in mice to investigate the antinociceptive property at the central and peripheral levesl. We observed a significant orexant e_ect for LONI11 and an intense anorexant e_ect for (S)-methyl 2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (LONI2) and LONI4. In zymosan-induced edema and hyperalgesia, LONI11 reduced the percent of paw volume increase and paw latency after s.c. administration, also suggesting a possible peripheral anti-inflammatory activity.

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# NEW CLASS OF POTENTIAL ANTIDIABESITY AGENTS TARGETING DPPIV AND CAs ENZYMES

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Type 2 diabetes (T2DM) is a chronic condition characterized by dysregulation of carbohydrate, lipid, and protein metabolism and results from impaired insulin secretion, insulin resistance, or a combination of both. Of the three major types of diabetes, T2DM is far more common (accounting for more than 90% of all cases) than either type 1 diabetes mellitus (T1DM) or gestational diabetes[1]. Globally, 6.28% of the world's population is affected by T2DM. Developed regions, such as Western Europe show higher prevalence rates that continue to rise despite the public health measures. The distribution of T2DM generally matches socio-economic development even if the burden of suffering due to diabetes is rapidly rising in lower income countries[2]. Thus, T2DM is recognized as a global public health concern which directly impacts on human life and health expenditures. Moreover, T2DM incidence has risen in lockstep with the obesity pandemic during the last half-century[3]. In fact, the numbers of individuals with T2DM parallel the numbers of adults with obesity generating a worldwide dual epidemic which is an important public health issue4. The detrimental health effects of diabetes and obesity are well-known so much that they are described by the term "diabesity". For these reasons and considering that the two conditions share key pathophysiological mechanisms, the management of patients with T2DM is shifting from cardio-centric goal to a new weight-centric, or, even better, adipose-centric treatment goal[4]. Given the relevance of Dipeptidyl Peptidase IV (DPP IV) and Carbonic Anhydrase (CA II and V) roles in the pathology of T2DM and in the weighting loss we herein report a new class of multitarget ligands addressed on the previously mentioned enzymes. The study moved from the observation that known inhibitors for these two enzymes share some key moieties which thus can be merged in a unique molecule endowed with a suitable scaffold to attain effective multiple inhibitions. The starting scaffold was identified by repurposing analyses focused on reported adrenergic ligands. The selected repurposed molecule was the known α1-AR inhibitor, WB-4101, which was progressively modified through a tailored morphing strategy to optimize the potency on DPP IV and CAs, while losing the adrenergic activity.

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# TOWARDS THE CHARACTERIZATION OF CORRECTOR ARN23765 MECHANISM OF ACTION VIA PHOTO-AFFINITY LABELING (PAL) APPROACH

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Cystic Fibrosis (CF) is a rare genetic disease characterized by deficiencies in the synthesis or function of the CF transmembrane conductance regulator (CFTR) anion channel, caused by mutations in the CFTR gene. Small-molecule compounds addressing the basic defect of the disease have been described, and are referred to as CFTR modulators. Among these, ***ARN23765***, a potent F508del-CFTR corrector discovered by our group, showed high potency in rescuing the function of mutant CFTR in primary human bronchial epithelial cells from F508del/F508del CF patients.[1]

CFTR correctors can act either directly, by binding to CFTR, or by interacting with the protein machinery responsible for CFTR synthesis and maturation. Generally, indications of correctors binding to CFTR derive from either indirect proofs or by experiments with purified wild type (wt) full-length protein or single domains.[2-3] No data are so far available disclosing the interaction of modulators with CFTR (either wt- or F508del mutant) in a native cellular environment.

To investigate the possible biological target(s) of ***ARN23765*** in living cells, a Photo-Affinity Labeling (PAL)[4-6] approach has been pursued as a convenient strategy to enable small molecule-protein trapping and subsequent target identification. To this purpose, several photo-affinity probes (PAPs) have been designed and synthesized by introducing a small photo-reactive moiety and a reporter/purification tag (or a chemical handle suitable for conjugation to such a tag) on the scaffold of ***ARN23765***.

Diazirine was selected as convenient photo-reactive small heterocyclic moiety, since its activation by UV light produces highly reactive transient chemical species that crosslink in a covalent manner to bio-molecules in close proximity.[5] On the other hand, suited tags or chemical handles were employed to allow probe-target adducts identification via different approaches (e.g., electrophoresis experiments, western blot, and mass spectrometry studies).

Notably, these chemical manipulations were applied in such a way as to produce minimal perturbations on the structure of the parent compound in order that novel synthesized PAPs could maintain bioactivity in a standard assay in CFBE41o- cells.

After a preliminary evaluation of their activity in rescuing F508del-CFTR function, novel PAPs were used in both cell lysates and intact cells demonstrating their in situ binding to wild type and mutant F508del-CFTR in CFBE41o- cells.

To the best of our knowledge, our study is the first to disclose the interaction of a corrector probe to wild type and mutant F508del-CFTR in an integral cellular setting.

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# NEW NICOTINAMIDE MIMIC SCAFFOLD ALLOWED NANOMOLAR INHIBITION OF HUMAN PARP ENZYMES

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The PARPs and tankyrases (TNKSs) form a family of 17 structurally and functionally diverse enzymes, which are involved in the regulation of various key biological and pathological processes such as DNA repair, cell differentiation, gene transcription, and signal transduction pathways.[1,2] These enzymes use $NAD^+$ to transfer an ADP-ribose unit onto target proteins or nucleic acids with a release of nicotinamide, and can catalyze both mono-ADP-ribosylation (mono-ARTs) or poly-ADP-ribosylation (poly-ARTs).
The discovery of synthetic lethality of the poly-ART PARP1 inhibition in the context of BRCA deficient cancer led to the first approved drug, Olaparib, in 2014.[3] Additional PARP inhibitors such as rucaparib, niraparib, and talazoparib were approved for use in various clinical settings more recently.
In contrast to poly-ARTs, mono-ART inhibitor development has lagged behind.[4]

Here, we report the identification of a set of compounds based on a new nicotinamide mimicking chemotype.[5] By varying substituents around the core, we were able to take advantage of distinct features of the conserved active sites of the PARP enzymes to rationalize the selectivity. We have identified analogs showing selectivity to poly-ADP-ribosylating PARP1, PARP2, and TNKSs as well as towards mono-ARTs such as PARP7, PARP11, PARP12, PARP14, and PARP15 with nM potencies; one compound emerged as the most potent PARP10 inhibitor described to date with an $IC_{50}$ of 7.8 nM.
The binding mode of the compounds was studied through their complex crystal structures with poly-ARTs (PARP2 and TNKS2), and mono-ARTs (PARP14 and PARP15).

The new scaffold does not possess inherent cell toxicity and by using PARP10 as an example we show that the inhibitors can enter cells and engage with the target protein. This, together with favorable ADME properties, demonstrates the potential of the scaffold for development of selective inhibitors against specific enzymes.

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# SPINDLIN-1 DEGRADERS: STAIRWAY TO HEAVEN (?)

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Histone post-translational modifications (PTMs) have been proposed to constitute a "histone code", which helps to organize genetic information at the chromatin level, and play a pivotal role in gene expression, cell differentiation, and development.[1] During the past decade, a wealth of "reader" modules have been characterized for histone PTM recognition. In particular, the detection of methylated histone tail lysine residues by Tudor domains plays important roles in epigenetic control of gene expression and DNA damage response. Among them, Spindlin1 (SPIN1) is a protein of the SPIN/SSTY family implicated in the regulation of gametogenesis.[3] Furthermore, its overexpression perturbs the cell cycle, induces chromosome instability, and leads to tumorigenesis,[4-6] even if the molecular mechanisms remain poorly understood.

Starting from a "library-on-library" screening approach, we recently identified the first-in-class chemical probes for the SPIN1 methyl-lysine reader domain.[7] Prompted by our interest in the discovery of promising alternatives to the traditional inhibition of epigenetic targets, a targeted protein degradation approach was used, in order to reduce the intracellular concentration of the protein of interest by inducing its proteolytic degradation. Here we report the design, synthesis, and cellular activity of a series of PROTACs (Proteolysis Targeting Chimeras) and in-cell click-proteolysis targeting chimeras (CLIPTACs), paving the way for future progress in Spindlin1 biology and its therapeutic applications (Figure 1).

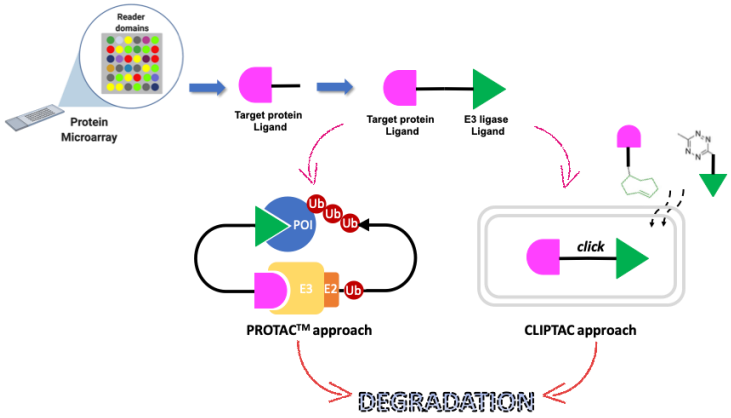


**Figure 1. Aim of the work**

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# BROAD SPECTRUM METALLO β-LACTAMASES INHIBITORS: NEW TOOLS AGAINST CLINICALLY-RELEVANT CARBAPENEMASES

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Bacterial multidrug resistance poses a real menace to public health, calling for new discoveries and innovation in antibiotic research.[1] Carbapenems are last resort antibiotics belonging to the most widely used family of β-lactam antibiotics. Their efficacy is currently challenged by the increasing prevalence of carbapenem-resistant clinical isolates, essentially Gram-negative bacteria producing peculiar β-lactamases (carbapenemase). Carbapenemases show a variable substrate profile and belong to molecular classes A (KPCs) and D (OXAs) of active serine β-lactamases or class B zinc-dependent metallo-β-lactamases (MBLs).[2] Among these, VIM- and NDM-type variants are the most widespread and have been identified in many bacterial species and are therefore recognized as a global health threat and critical drug targets. Efficacious inhibitors for MBLs are still missing, in fact, many MBLs are able to hydrolyse even the last resort antibiotics.

In a previously multidisciplinary approach integrating in silico/in vitro analysis for the design and identification of novel non-covalent β-lactamase inhibitors[2] we identified 4H-1,2,4-triazole-3-thiol as a promising scaffold of NDM-1 inhibitors, and thiosemicarbazone derivatives as potential broad-spectrum inhibitors, i.e. active on both serine- and metallo-β-lactamases.[3]

Here we report on the optimization of 4H-1,2,4-triazole-3-thiol[4] and thiosemicarbazone inhibitors, through the synthesis of new analogues carrying substituents with different electronic features, their in vitro biological activity, as well as in silico simulations and X-ray crystallography analysis of complexes, allowing a better understating of their mechanism of inhibition.

Among 4H-1,2,4-triazole-3-thiol derivatives, the best compounds were able to inhibit both class A and class B enzymes and to significantly potentiate the activity of meropenem in in vitro antibiotic susceptibility assays. Similarly, screening a library of thiosemicarbazone derivatives, we identified compounds inhibiting NDM-1 in the sub-micromolar range and CTX-M-15 at low micromolar level, and showing up to 500-fold reduction of meropenem MIC, when tested on NDM-1-producing strains. Most interestingly, some of the above compounds also showed a direct-acting antibacterial activity against different Gram-positive and Gram-negative clinical isolates showing various antibiotic-resistance phenotypes.

These results suggest that the new identified compounds might be promising leads for the development of new dual-acting broad-spectrum β-lactamase inhibitors, critically needed in the fight towards antibiotic resistance.

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# NOVEL DIPEPTIDE NITRILES AS ANTITRYPANOSOMAL AGENTS TARGETING RHODESAIN OF *TRYPANOSOMA BRUCEI RHODESIENSE:* DEVELOPMENT AND COMBINATION STUDIES

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Human African Trypanosomiasis (HAT) is caused by protozoa of *Trypanosoma genus*, with two subspecies able to induce the disease in humans: *T. brucei gambiense* is responsible for a chronic form of HAT, while *T. brucei rhodesiense* is able to cause the acute lethal form of HAT, with a high mortality rate.[1]
Current HAT therapy is based on dated drugs with a limited spectrum of action, toxicity and problems related to the parenteral route of administration. Recently, fexinidazole was introduced in therapy for oral administration, thus improving patient compliance; however, its action is limited to the gambiense form of HAT. In the present scenario, rhodesain, the main cysteine protease of *T. brucei rhodesiense*, is considered a promising target for the drug-discovery process of the rhodesiense lethal form of HAT.[2]
Our research team has been involved in the last fifteen years into the development of novel antitrypanosomal agents targeting rhodesain. In our more recent work, we synthesized novel dipeptide nitriles **1-2a-e** to explore the reactivity of the novel warhead, considering that literature data suggest the strong ability of this electrophilic moiety to react with the catalytic cysteine of rhodesain.
In the designed derivatives, the P1 site bears a homophenylalanine, a critical residue for the affinity towards the target enzyme, while Phe and Leu residues, normally preferred by rhodesain, were sampled at the P2 site. The amino group of the P2 substituent was protected with a fluorobenzoyl substituent, spanning into P3 region, to optimize the interactions of the fluorine atom/s with the S3 pocket.
Within the most interesting compounds, the dipeptide nitrile **1b** showed the highest binding affinity towards rhodesain ($K_i$=16 nM), coupled with a good antiparasitic activity ($EC_{50}$=10.1 µM). We also proved that nitrile **1b** directly binds to the active site of rhodesain, acting as competitive inhibitor.[3]
Finally, we carried out a combination study, according to Chou and Talalay method, on the novel identified lead compound **1b** with curcumin, a golden multitarget nutraceutical obtained from *Curcuma longa* L., which we demonstrated to inhibit rhodesain in a non-competitive manner. In this combination study we obtained a Combination Index < 1 for the most relevant affected fractions ($f_a$), thus suggesting that the dipeptide nitrile **1b** and curcumin synergistically inhibit rhodesain, with an antitrypanosomal activity of the combination of the two inhibitors expressed by an $EC_{50}$=4.85 µM.

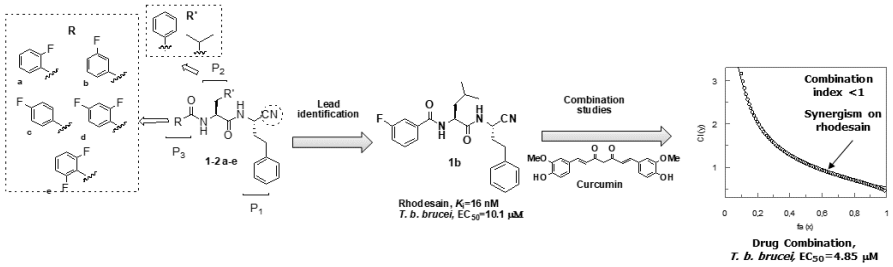


**Figure 1.** Structures of novel dipeptide nitriles **1-2a-e** and combination studies with curcumin

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# TRPM8 ION CHANNEL: NEW TARGET IN THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER (CRPC)

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Prostate cancer (PC) is the second most frequently diagnosed cancer in men and several therapeutic approaches are currently available for patients. Nevertheless, PC might escape the treatments, giving rise to more aggressive forms of cancer. At this stage, PC often spreads, with a significant increase in the mortality rate.[1] TRPM8 gene was first identified as a reporter of the androgen receptor (AR) transcriptional activity in PC cells[2] and subsequently proposed as a PC biomarker. Its expression, indeed, increases during the initial stages of the disease, declining after androgen deprivation therapy.[3] Although TRPM8 expression depends on AR transcriptional activity,[4] biochemical findings have reported a direct TRPM8 interaction with androgens or androgen receptor (AR).[5,6] So, TRPM8 has become a druggable target in prostate cancer and TRPM8 modulators have been proposed as potential anticancer agents in this pathology. In the last years, our research group has contributed to the discovery of new chemotypes as TRPM8 modulators and, furthermore, we recently investigated the effects exhibited by TRPM8 modulators in different kinds of PC-derived cell lines. We demonstrated in 2D and 3D models of AR+-PC cells, including the CRPC cells, the effectiveness of TRPM8 antagonists, in reducing tumor proliferation, migration, and invasiveness. Our research also revealed the involvement of the AR pathway in the efficacy of TRPM8 antagonists as anticancer agents, relying on a non-genomic pathway.[7] Given our previous results, in the present work we describe the design and the synthesis of a new series of TRPM8 antagonists. To this aim, fluorimetric calcium assays have been used for the preliminary assessment of synthesized compounds potency and selectivity. The preliminary screening allowed the identification of several potent (0.11 μM < $IC_{50}$ < 0.49 μM) and selective compounds. The most potent derivatives were further characterized by patch-clamp electrophysiology assays, confirming their interesting activity. Moreover, the behavior of these compounds was investigated in 2D and 3D models of PC. These TRPM8 antagonists showed remarkable efficacy in inhibiting the growth induced by androgen in various PC cells as well as in CRPC models, confirming their potential as anti-prostate cancer agents.

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# CHALLENGE TRANSABILITY OF "IN VITRO" TO "IN VIVO": GENE-EXPRESSION BIOMARKERS AND FLUORESCENT IMAGE-GUIDED SURGERY PROBES IDENTIFICATION FOR OVARIAN CANCER

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Ovarian cancer is the second most prevalent gynecologic malignancy, and the Ovarian Serous Cystadenocarcinoma (OSCA) is the most common and lethal subtype of ovarian cancer.[1] Current screening methods have strong limits in early detection and, despite surgery and chemotherapy, the majority of OSCA patients relapse and eventually succumb to their disease, suggesting that further efforts are required to improve early diagnosis.
Furthermore, the identification and removal of all gross and microscopic tumor to render the patient disease free represents a huge challenge in ovarian cancer treatment. The presence of residual disease is an independent negative prognostic factor.
In this presentation, it will be described the development and cross-validated method for detecting gene-expression biomarkers able to discriminate OSCA tissues from healthy ovarian tissues and from other cancer types with very high accuracy.[2]
Then, it will be described the synthesis (Figure), "in vitro" and "in vivo xenograft model" evaluation of *ad hoc* developed probes for fluorescent image-guided surgery, particularly useful in the resection of not palpable and not visible at naked eye lesions.[3,4]

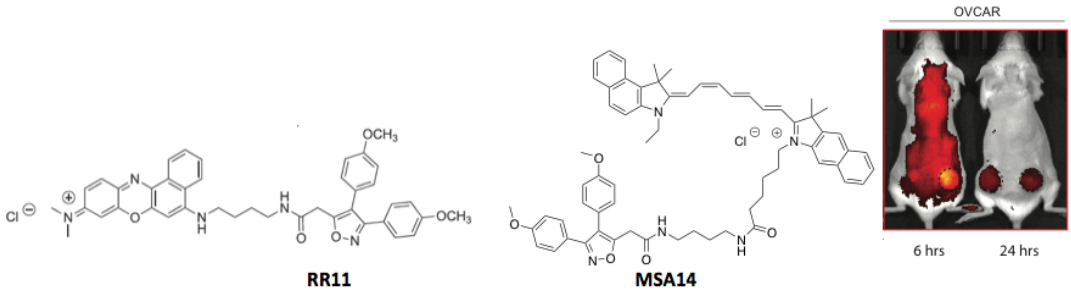


**Figure.** Chemical structure of florescent probes and image of RR11 uptake in ovarian cancer developed by xenograft mice treated human ovarian cancer OVCAR cells.

The probes bearing fluorochromes with different fluorescent properties were used to challenge the transability of "in vitro "results to "in vivo" pre-cilinical model of ovarian cancer built by implanted human ovarian cancer cells in mice.[3]

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OC-15

# THE PADAM OXIDATION ROUTE FOR THE SYNTHESIS OF SARS-CoV-2 MAIN PROTEASE INHIBITORS

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In 2019, SARS-CoV-2 caused worldwide the current outbreak named COVID-19. Despite multiple countermeasures implemented and approved DNA, RNA and protein subunits vaccines an additional step forward has been made thanks to approved drugs targeting the CoV RdRp (e.g. Remdesivir i.v., Molnupiravir p.o.) and the CoV 3CL Protease (Nirmatrelvir) although they suffer of modest efficacy or suboptimal PK properties. It is an urgent global need to identify new direct-acting antiviral drugs (DAAs) against this pathogen and new emerging viruses, in order to prevent the progression to severe disease or new pandemic. In particular, the main protease ($M^{pro}$) of SARS-CoV-2 is a cysteine protease playing the essential role in viral replication, thus being identified as a solid target for the development of effective antiviral drugs.[1] The knowledge of both catalytic mechanism and substrate specificity of the $M^{pro}$ triggered the idea to exploit multicomponent reactions (MCRs) as a fast and versatile synthetic tool toward novel $M^{pro}$ inhibitors. Accordingly, the Passerini reaction-amine deprotection-acyl migration (PADAM) oxidation route, was employed for the development of novel small peptidomimetic compounds with a ketoamide warhead behaving as covalent reversible inhibitors (Figure 1).[2] The peptidomimetics prepared were evaluated in SARS-CoV-2 $M^{pro}$ biochemical and antiviral cell-based assays, showing $IC_{50}$ and $EC_{50}$ in nanomolar / low micromolar range. Furthermore, X-ray co-crystal structures of protease–inhibitor complexes were determined as a part of this study, revealing the molecular determinants of the interaction with the $M^{pro}$ and providing key hints for further optimization.

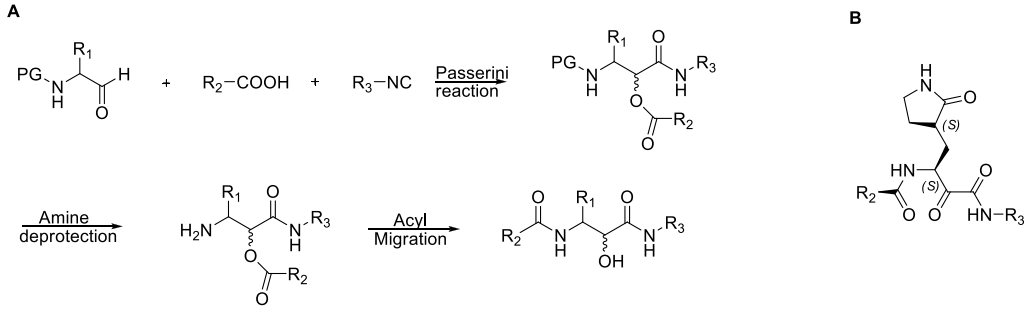


**Figure 1. A**. The PADAM strategy for the α-hydroxy-β-amino amides. **B**. General structure of our synthesized α-ketoamides.

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OC-16

# DISCOVERY OF DIKETO ACID DERIVATIVES TARGETING THE SARS-CoV-2 NSP13 HELICASE

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The severe and acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a plus single-strand RNA β-coronavirus belonging to Coronaviridae, closely related to Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1). This virus is responsible for an ongoing global pandemic of the disease namely COVID-19 that caused many deaths in the human population. Because of this, it is imperative to find drugs able to solve this global health issue as fast as possible. The development of vaccines is essential in the containment of the diffusion of the virus, and an incredible joint effort led to a global vaccination campaign in about 1 year after the COVID-19 outbreak. However, vaccines may be less or no effective against emerging variants of SARS-CoV-2 and, also, it is still unknown how long this vaccine-induced immunity will last. Therefore, the development of antiviral drugs against SARS-CoV-2 is of pivotal importance.

The SARS-CoV-2 non-structural protein 13 (nsp13) has been identified as a target for antiviral drugs thanks to its critical role in viral replication and to its high sequence conservation within the coronavirus family. Indeed, the amino acid sequence of SARS-CoV nsp13 and SARS-CoV-2 nsp13 shares a sequence identity of 99.8% and a sequence similarity of 100.0%, with only a single amino acid difference between nsp13s of SARS-CoV (I570) and SARS-CoV-2 (V570).[1] Nsp13 is a member of the SF1B helicase family, targeting the natural nucleotides and deoxynucleotides as substrates when performing its adenosine triphosphatase (ATPase) activity. Nsp13, in fact, utilizes the energy of nucleotide triphosphate hydrolysis to catalyze the unwinding of double-stranded DNA or RNA in a 5' to 3' direction. Besides its helicase activity, nsp13 also possesses RNA 5' triphosphatase activity, suggesting a further essential role in the formation of the viral 5' mRNA cap.[2]

Although the roles of nsp13 in the viral lifecycle, there is a paucity of information about small molecules compounds reported in literature endowed with nsp13-inhibitory activity. Aryl diketo acids (DKAs) have been previously described as inhibitors of nsp13 of SARS-CoV-1.[3] Basing on these literature data and thanks to our longstanding expertise in the design and synthesis of DKA derivatives, we carried out a semi-random screening on our in-house library of DKA compounds with the aim to identify new effective compounds endowed with inhibitory activity against nsp13. In particular, we found a set of our in-house indolyl DKA derivatives as new SARS-CoV-2 nsp13 helicase inhibitors. Also, we designed and synthesized a new series of analogues structurally related to the identified inhibitors. The data coming from the biological assays will be shown and discussed.

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# AN INTEGRATED MEDICINAL CHEMISTRY WORKFLOW FOR THE DEVELOPMENT OF NEW PEPTIDES AS SARS-COV-2 MPRO COVALENT INHIBITORS

Musella, S.;[a] Sala, M.;[a] Di Micco, S.;[b] Di Matteo, F.;[a] Vivenzio, G.;[a] Andrei, G.;[c] Snoeck, R.;[c] Marquez, J. A.; [d] Rahimova, R.;[d] Fasano, A.[e,b] and Campiglia, P.;[a]

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The outbreak of novel coronavirus disease caused by the pathogen SARS-CoV-2 has resulted in over 61.8 million infections and over 1.4 million deaths worldwide. These reported estimations highlight the need for the development of antiviral therapies to tackle this deadly virus. Despite vaccines play a pivotal role in the fight against COVID-19 pandemic, antiviral drugs should provide many distinct advantages while maintaining a complementary approach.[1] Therefore, it is evident that discovery of small molecules and peptidomimetics as anti-SARS-CoV2 agents represent a valid alternative approach to expanding anti-SARS-CoV-2 therapeutic arsenal. We recently demonstrated that the zonulin inhibitor AT1001 (Larazotide acetate),[2] currently in phase 3 trials in celiac disease, binds the Mpro catalytic domain. Specifically, we observed that it shares a similar structural pattern to the peptidomimetic inhibitor N3 and 13b.[3] These structural motifs led to the development of a new rational and ambitious research program act to investigate AT1001 derivatives as potential new inhibitors of $M^{pro}$ enzyme.[4]

Based on previous results concerning AT1001 analogues, we collected pivotal clues to design a new series of more potent $M^{pro}$ covalent inhibitors. In the present work, we discuss a chemical workflow process leading to the development of a new series of reversible covalent tripeptide derivatives from the lead compound AT1001.

Driven by in silico approach, we designed a small library of tripeptides bearing different structural modifications. $M^{pro}$ inhibitory activity of the synthesized peptides was evaluated by FRET methods. The experimental workflow led to the identification of a new hit compound (**5**) showing an activity profile comparable to calpeptin, a known reversible covalent inhibitor of Mpro used as reference compound.[5] Using X ray crystallography, we demonstrated the covalent bond formation between compound **5** and cys145 of $M^{pro}$. Starting from compound **5** a hit-to-lead development process was carried out, leading to the identification of compounds **43** and **44**. These modifications have increased lipophilicity, while preserving the binding properties towards the $M^{pro}$, thus facilitating the penetration of peptides across biological membranes and improving pharmacokinetic properties. Computational studies predicted compound **44** (Ac-GGVLVQPG-$NH_2$) as the most membrane-permeable derivatives. These results justify the micromolar range antiviral activity showed by compound **44** ($EC_{50}$ = 17.6 ± 2.4 µM), in a cellular model of SARS-CoV-2 infection, using Vero cells transfected with two different clinical isolated SARS-CoV-2 strains.

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OC-18

# DEVELOPMENT OF NOVEL ENZYME INHIBITORS OF THE ENDOCANNABINOIDS' CATABOLISM FOR THE TREATMENT OF EPILEPSY AND NEUROINFLAMMATORY CONDITIONS

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The main endocannabinoids (ECs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG), by stimulating cannabinoid CB1 and CB2 receptors (CB1R and CB2R), regulate relevant signaling pathways. The key enzymes involved in ECs catabolism are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Their unique role in terminating ECs signaling and regulating the intracellular levels of AEA, 2-AG and other ECs supports their potential as therapeutic targets. Selective or dual inactivation of ECs degrading enzymes represents an attractive approach for eliciting the desirable effects of CBR activation, while avoiding the negative (psychotropic, among others) effects of CB1R stimulation.
Epilepsy is the second most common neurological disorder with an incidence rate of 0.3-0.5% worldwide. Most of conventional anti-epileptic drugs have narrow therapeutic margin and require therapeutic drug monitoring. Despite the introduction of many II-generation anti-epileptic drugs, pharmacoresistant epilepsy has not been significantly reduced. A reduction of AEA in patients affected by temporal lobe epilepsy (TLE) has been clearly documented. Similarly, the neuroprotective role of AEA was confirmed by the kainic acid induced increase of AEA in hippocampus, which, without affecting 2-AG, provides "on demand" protection against acute excitotoxicity. Seizure activity at the cellular level initiates significant influx of calcium. Over time, impaired neuronal calcium homeostasis increases the activity of pro-oxidant cellular systems. The enocannabinoid system can delay or prevent excitotoxic damage by re-balancing the main excitatory/inhibitory systems and directly modulating mitochondrial function, thus preventing oxidative stress–related epileptogenesis. This rationale led to the proposal of FAAH inhibitors as therapeutic option for the prevention of epileptic disorders. To strengthen this hypothesis we demonstrated that our potent and selective FAAH inhibitor (NF1245, Ki=160 pM [1]) ameliorated the acute epileptic behavior and prevented hippocampal oxidative damage in rat model of pilocarpine-induced epilepsy when tested at 10 mg/kg.[2] The same dose was also effective in TLE generated by electric kindling. Based on our recent results,[3] rational structural modifications produced improved analogues that were able to reduce the oxinflammation state by decreasing DNA-binding activity of NF-kB p65, devoid of cytotoxic effect and unwanted cardiac effects while being able to reduce the severity of the pilocarpine-induced status epilepticus. [2]
Neuro-inflammation, mainly regulated by microglia and astrocytes, is a common feature of several neurodegenerative diseases amyotrophic lateral sclerosis, and multiple sclerosis. In fact, neurological disorders can be triggered by chronic neuro-inflammatory conditions that, through different mechanisms in which the oxidative stress is involved, may lead to neuronal cell death. Oxidative stress is caused by increased levels of reactive oxygen species (ROS) deriving from mitochondrial dysfunctions. Elevating endogenous levels of the ECs through FAAH and MAGL inhibition is a valuable approach to enhance the anti-inflammatory, anti-nociceptive [4, 5] and neuroprotective effects mediated by the ECs. On these bases, we have recently designed a new library of FAAH and MAGL inhibitors of nanomolar potency embedding ionizable functions, for improving water solubility and the chemical stability. For these compounds, effective in reducing ROS production in 1321N1 astrocytes, the anti-inflammatory activity was evaluated ex vivo in hippocampal slice cultures.

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# HIJACKING THE FOLDING PROCESS FOR TARGETED PROTEIN DEGRADATION

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Targeted protein degradation (TPD) is an emerging and powerful tool to target disease-causing proteins difficult to hit with traditional small molecule inhibitors. The approach is based on inducing protein degradation by using a specific molecule, called degrader, that promotes the selective redirection of the bound protein to the protein degradation pathways.[1]

Very recently, we have proposed a novel drug discovery approach for the rational identification of non-conventional small molecule degraders acting on protein folding process. This novel class of compounds lowers the protein levels of a target of interest preventing it from reaching the native state. We refer to this strategy as Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT).[2]

The PPI-FIT approach was applied to target the prion protein (PrP) a cell surface glycoprotein playing a pivotal role in fatal and incurable neurodegenerative disorders known as prion diseases. Despite considerable drug discovery efforts to identify effective anti-prion agents, prion diseases currently represent an unmet medical need. [3] The absence of obvious biologically relevant binding sites and a large amount of negative screening data ultimately lend further support to the idea that PrP may simply belong to the class of proteins classified as "undruggable" by canonical drug discovery protocols.[4]

In our study, the reconstruction of the PrP folding pathway through all-atoms MD simulation allowed the identification of a metastable intermediate of the PrP folding pathway characterized by a druggable pocket. Virtual screening of a commercial small molecule library resulted in the identification of thirteen potential binders, four of which capable of selectively lowering the load of PrP into the cellular membrane and promote its degradation.

The most interesting compound SM875 ($IC_{50}$ = 7.87 ± 1.17 µM) was able to i) reduce PrP loads in several different cell lines, either expressing the protein endogenously or exogenously, without decreasing PrP mRNA, ii) selectively promote the degradation of nascent PrP molecules by the autophagy-lysosomal pathway, while the molecule neither binds nor exerts any effect on mature cell-surface PrP, and iii) exhibit an dose dependent anti-prion activity in prion-infected mouse fibroblasts. Collectively, these data provided strong experimental support for the notion that SM875 acts by targeting a folding intermediate of PrP and paving the way to hit-to-lead optimization efforts.

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OC-20

# THE PIVOTAL ROLE OF PYRROLIDINE RING AS MULTITARGET SCAFFOLD IN NEURODEGENERATIVE DISEASES

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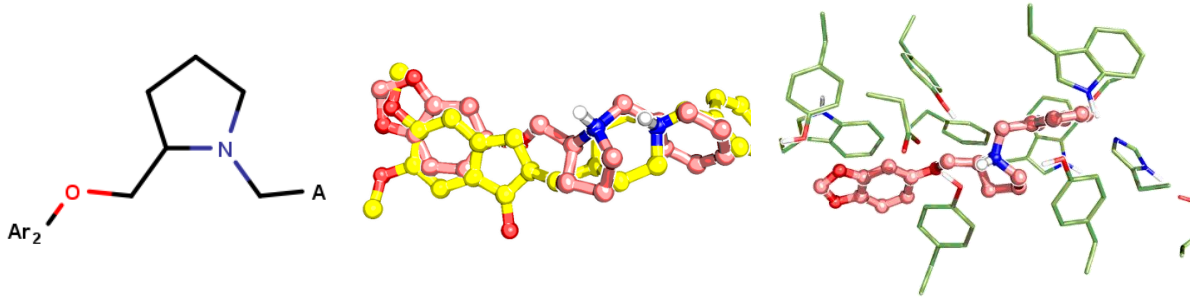
In pharmaceutical science and drug design the versatility of the pyrrolidine scaffold in rapport to spatial arrangement, synthetic accessibility and pharmacological profile is a largely explored and most likely interesting one.[1] In this scenario, our previous study reported the design and synthesis of a series of *N*-substituted aryloxymethyl pyrrolidines as enantiopure chiral compounds exploiting the stereochemical requirements for P-gp binding.[2] We then challenged the enrolling in the field of Alzheimer disease of so far not ravelled targets of this chemical cliché with a structure based and computer-aided design strategy focusing on multi-target action, versatile synthesis as well as pharmacological safeness. To achieve these hits, novel derivatives were obtained and tested, and the biological data will be here presented and discussed.

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# PURSUING THE COMPLEXITY OF BIPOLAR DISORDER: RATIONAL DESIGN AND OPTIMIZATION OF FIRST-IN-CLASS D3R/GSK-3β MODULATORS TOWARDS AN IN VIVO PROOF OF CONCEPT

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Bipolar disorder (BD) is a complex neuropsychiatric condition, in which the same patient can exhibit alternating episodes of mania and depression.[1,2] Since molecular details of BD etiopathology remain rather controversial,[3] fully validated *in vivo* models capable of emulating cycles/switches between disorder states are still missing.[4] BD represents a global unmet medical need that lacks disease-modifying and multimodal treatments.

We attempted to overcome the limitations of single-target drugs by devising multitarget-directed ligands (MTDLs)[5] to simultaneously modulate dopamine D3 receptor (D3R) and glycogen synthase kinase-3β (GSK-3β), two structurally unrelated targets that play independent, yet connected, roles in cognition and mood regulation.[6,7]

Combining computer-aided drug design, synthetic efforts, and *in vitro* pharmacology, we discovered **ARN24161** that bears a 2-oxo-6-(3-pyridyl)-3*H*-benzimidazole-1-carboxamide moiety directed to both the D3R specificity binding pocket (SBP) and GSK-3β hinge region and a 2,3-dichlorophenylpiperazine to target ASP110 in the orthosteric binding pocket (OBP) of D3R. **ARN24161** is a promising prototype endowed with a partial agonist profile at D3R in the low nanomolar range and high nanomolar inhibition of GSK-3β and showed acceptable drug-like properties in *in vitro* stability and PK studies.[8]

A combination of computational predictions and crystallographic studies on GSK-3β led to a rational SAR exploration of the chemical space around **ARN24161**. We were able to boost the affinity for the enzyme and to improve its *in vitro* ADME properties and PK profile. Furthermore, a Mass-MetaSite approach was applied for metabolites analysis[9] in mouse liver microsomes of some D3R partial agonists/GSK-3β inhibitors.

Eventually, some well-balanced lead compounds endowed with improved drug-like properties have been selected for further PK and BBB permeability studies, with the ultimate objective of investigating their antipsychotics effects in behavioral studies on mice.

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# S.M.A.R.T. STEROIDS: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDY TOWARDS ALLOSTERIC MODULATORS OF N-METHYL-D-ASPARTATE RECEPTORS

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Neurosteroids are compounds synthesized in the nervous tissue from cholesterol or steroidal precursors from peripheral sources. It is believed that neurosteroids execute their effects by modulating the activity of different membrane receptors, including the glutamatergic ionotropic receptors, e.g. N-methyl-D-aspartate receptors (NMDARs). The NMDARs play an important role in development, synaptic plasticity, learning, and memory; however, abnormal activation of NMDA receptors have been shown to mediate neuronal degeneration/cell death. To find novel potentially beneficial drugs to treat neurological damage or neurodegeneration is one of the most investigated areas in contemporary pharmacology and neuroscience. Therefore, we have designed and synthesized a library of SMART Steroids – **S**teroidal **M**olecules **A**s **R**apid-acting **T**herapeutics. Our SMART steroids are neuroactive molecules, acting as positive or negative allosteric modulators of NMDARs depending on their structure. The *in vitro* effect is evaluated by manual patch-clamp technique on human embryonic kidney cells (HEK293) transfected with plasmids encoding GluN1-a/GluN2B/GFP genes assessing NMDAR inhibition[1-4] or potentiation[5] rates and IC50/EC50 values. Next, our ADMET screening pipeline[6,7] currently covers physicochemical and biological properties like: *(i)* solubility; *(ii)* Caco-2 and PAMPA permeability; *(iii)* plasma and microsomal stability; *(iv)* pharmacokinetic profile. Then, our compounds are tested in a series of experiment on neuronal cultures assessing neuroprotection against glutamate and NMDA-induced neurotoxicity (survival rate, caspase-3, intracellular calcium levels, ROS).[8,9] Finally, our compounds are tested *in vivo* targeting desired effect including the models of animal behavior (open field, elevated plus maze, forced swim test, etc.).[10,11]
Our results indicate that these compounds do afford neuroprotective effect and as such, SMART steroids may be beneficial in the treatment of several neurological diseases. A broad patent portfolio has been developed protecting compounds, production and its use for treatment in neurology etc. (US 10,017,535; CZ 307648; US 8575376; EP 2675821).
For details, see https://kudova.group.uochb.cz/en

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# NUCLEIC ACID APTAMERS: POTENTIAL THERAPEUTIC AGENTS FOR CANCER AND NEURODEGENERATIVE DISORDERS

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Nucleic acid aptamers are innovative and promising candidates for therapeutic and diagnostic applications. Also known as chemical antibodies, they fold into peculiar three-dimensional structures capable of binding specific targets with high affinity, thus inhibiting their function.[1] Aptamers exhibit significant advantages relative to protein therapeutics in terms of size, non-immunogenicity, manufacturing cost, synthetic accessibility, and modification by medicinal chemistry. Indeed, they can be easily modified and engineered into aptamer−drug conjugates and targeted delivery materials, increasing their therapeutic applications. The first aptamer approved for a therapeutic application was pegaptanib (Macugen, Pfizer), which was approved by FDA in 2004.[2] Nine RNA and five DNA aptamers are currently being evaluated in advanced clinical trials for several diseases, including cancer and aging-related disorders.[3]

AS1411 is a first-in-class anti-nucleolin aptamer with cancer-selective antiproliferative activity in human.[4] The mechanism of AS1411 action of is still under debate and multiple nucleolin-dependent and independent biological effects have been described.[5] AS1411 folds into a noncanonical DNA structure, termed G-quadruplex (G4), which makes it more stable against the serum nucleases and increases cellular uptake efficacy. With the aim of optimizing AS1411 structural features to find aptamers with improved anticancer properties, a small library of AS1411 derivatives has been designed, synthesized, and investigated for their enzymatic resistance in serum and nuclear extract, molecular binding mechanism, and ability to affect the viability of MCF-7 human breast adenocarcinoma cells. Most of them showed better antiproliferative activity on MCF-7 cells than AS1411 despite weaker binding to nucleolin, supporting the hypothesis that the antiproliferative properties of G4-forming aptamers are due to multi-targeted effects.[6]

Moreover, some G4-forming RNA aptamers showed a high affinity for the physiologically present cellular form of the prion protein ($PrP^{C}$) and a remarkable ability to prevent the hallmark event in the prion diseases, namely its conversion into the pathological form ($PrP^{Sc}$).[7] Such anti-prion aptamers containing four- or eight-tandem repeats of the r(GGA) sequence (R12 and R24, respectively) need chemical modifications for effective therapeutic activity. In this frame, the rational design, synthesis, and characterization of chemically modified R12 and R24 derivatives will be discussed. The results show the possibility of enhancing anti-prion aptamer properties through straightforward modifications, thus paving the way for the development of effective next-generation aptamer-based drugs against prion diseases.[8]

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OC-24

# COMBINING QUANTUM MECHANICS AND MACHINE LEARNING IN THE SEARCH OF THE BIOACTIVE CONFORMATION OF DRUG-LIKE COMPOUNDS

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In the last years, Machine Learning (ML) has emerged as a promising tool for an efficient calculation of molecular properties and chemical reactivity. ML techniques may alleviate the computational burden required to obtain an accurate description from high-level quantum mechanical (QM) computations. As an example, we limit ourselves to quote the series of ML ANI methods developed by Roitberg and coworkers to predict the total energies of organic molecules containing four atom type: H, C, N and O,[1,2] and subsequently extended to molecules containing F, Cl and S atoms.

In this talk, our aim is to present the results of a comparative analysis about the suitability of ANI methods to explore the bioactive species of drug-like compounds, which is dictated by a subtle balance between ionization, tautomerism and conformational preferences of flexible molecules. Our approach to solve this challenging question has relied on the definition of the Multilevel Strategy,[3,4] which combines a low-level sampling method to explore the conformational space of drug-like compounds (generally resorting to semiempirical QM methods or Molecular Dynamics simulations), and a high-level QM approach to estimate with chemical accuracy the relative stability of the main conformational species (generally involving MP2. Calculations with extended basis set in conjunction with solvation effects via the IEFPCM-MST continuum solvation model). Though the Multilevel Strategy has proven valuable for the analysis of the bioactive species in a variety of molecular systems,[5] the main limitation is the sizable computational cost, which could be significantly reduced through ML techniques. A critical evaluation of the implementation of QM/ML techniques will be presented.

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OC-25

# CHALLENGING BIOISOSTERIC SWITCH IN AChE-MAO B DUAL-TARGETING HIT OPTIMIZATION

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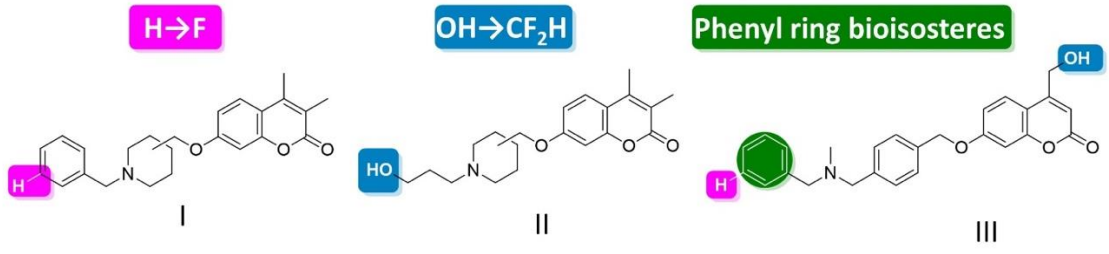
The development of multitargeting ligands represents a fascinating field of research that aims, among others, at providing disease-modifying drugs against neurodegenerative diseases, such as Alzheimer's disease (AD), that still lacks a resolving treatment. Over the last decade, we have developed diverse dual AChE-MAO B inhibitors as anti-AD probes by decorating a coumarin template.[1] Indeed these efforts provided promising hit compounds (Figure 1) endowed with potent in vitro profiles of target's modulation albeit showing sub-optimal drug-like features (e.g., high lipophilicity, low aqueous solubility). To overcome these drawbacks, different types of (bio)isosteric switches have been envisaged.




| | I (1,3) | I (1,4) | II (1,3) | II (1,4) | III |
|---|---|---|---|---|---|
| $IC_{50}$ h AChE | 1.0 μM | 1.7 μM | 1.8 μM | 1.2 μM | 0.42 μM |
| $IC_{50}$ h MAO B | 0.030 μM | 0.11 μM | 1.5 μM | 0.48 μM | 0.010 μM |

**Figure 1.** Chemical structure and biological data of starting hits. Designed bioisosteric replacements are underlined with different color codes.

In particular, by following a ligand-based approach we investigated classic H/F exchange and the replacement of alcohol-OH with a lipophilic hydrogen bond donor group ($CF_2H$).[2,3] Moreover, the availability of the X-ray complexes for compound **III** with both target enzymes (mouse AChE and human MAO B) guided the study of phenyl ring bioisosteres[4] along a structure-based design strategy.[5] The effect of bioisosteric replacement was assessed by focusing on both in vitro enzymatic activities and, preliminarily, early-ADME features (solubility, permeability, metabolic stability).

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OC-26

# EXPLORING CCRL2 CHEMERIN BINDING USING ACCELERATED MOLECULAR DYNAMICS

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Chemokine (C–C motif) receptor-like 2 (CCRL2), is a seven transmembrane receptor closely related to the chemokine receptors CCR1, CCR2, CCR3, and CCR5[(1)]. Nevertheless, CCRL2 is unable to activate conventional G-protein dependent signaling and to induce cell directional migration. The only commonly accepted CCRL2 ligand is the nonchemokine chemotactic protein chemerin (RARRES2)[(2)]. The chemerin binding to CCLR2 induce leukocyte chemotaxis and genetic targeting of CCRL2 was shown to modulate the inflammatory response in different experimental models[(3)]. This mechanism was shown to be crucial for lung dendritic cell migration, neutrophil recruitment, and Natural Killer cell-dependent immune surveillance in lung cancer. To gain more insight in the interactions involved in the CCRL2-chemerin, the binding complexes were generated by protein–protein docking, then submitted to accelerated molecular dynamics. The obtained trajectories were inspected by principal component analyses followed by kernel density estimation to identify the ligand-receptor regions most frequently involved in the binding. By visual inspection, the studied conformations were grouped into two general chemerin binding modes named BM1 and BM2 (Fig. 1 and 3); also, for the BM1, two patterns of interactions were observed (Fig. 1 and 2). To conclude, the reported analyses led to the identification of the putative hot-spot residues involved in CCRL2-chemerin binding.

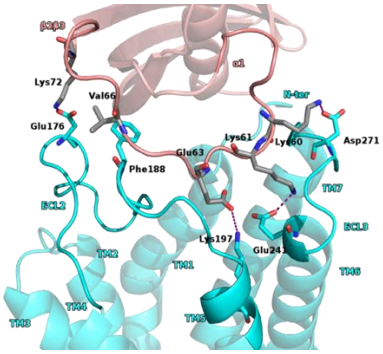


**Fig. 1 BM1 first pattern of interactions**

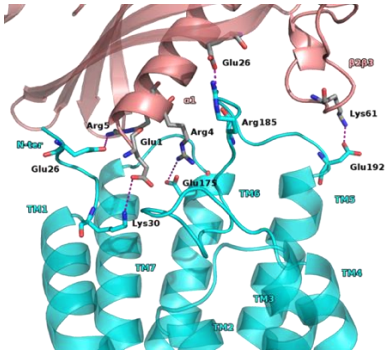


**Fig. 2 BM1 second pattern of interactions**

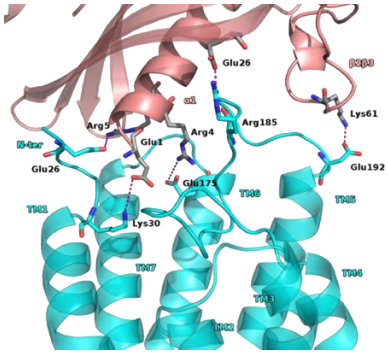


**Fig. 3 Proposed interactions for BM2**

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# FUNCTIONALIZED LIGANDS TARGETING G PROTEIN-COUPLED ADENOSINE RECEPTORS

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Functionalization of ligands for a specific target has different aims that include target probing, drug delivery, theranostic and bitopic or multitarget pharmacology. Adenosine receptors (ARs) belong to family A of G protein-coupled receptors (GPCR) and four different subtypes are present in humans: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$. AR subtypes are intracellularly coupled with different G proteins that, along with their peculiar distribution, give them a wide variety of implications in both physiological and pathological conditions. In particular, $A_3$AR is overexpressed in inflammatory and cancer cells, thus making it a potential target for therapy.[1] Over the years, our research group has developed highly potent and selective $A_3$AR antagonists that has served as the base to develop functionalized congeners.[2-5] In fact, development of a functionalized ligands involves the use of a pharmacophore which is conjugated to a function -bearing moiety by means of a linker. Thus, ligand have to present a covalently modifiable functional group (i.e. amino, hydroxy, carboxylic acid, iso(thio)cyanate, azide or alkyne groups). The orientation of the conjugable moiety in the GPCR binding site is of extremely importance: if it is oriented towards the inner part of the receptor, the conjugate could not bind the receptor with the same optimal pose, leading to a decrease of affinity and selectivity. In fact, the conjugable moiety has to point towards a solvent exposed part of the receptor oriented to the extracellular side of the membrane. For this reason, a deep knowledge of the pharmacophore structure–activity relationship is needed.

In our group, taking advantage from our expertise, we have developed various $A_3$AR antagonists bearing different conjugable moieties and investigating the effect of the linker. We have reported also some possible application such as the development of fluorescent and magnetic probes.[2-5] While fluorescent probes could be useful both for more classical drug discovery and diagnostic purposes, magnetic probes are quite new objects that could serve for separation of biological species, drug-targeting and/or delivery, imaging, and localized magnetic fluid hyperthermia-based treatments. In our case, we have developed $A_3$AR ligands conjugated to magnetic carbon nanotubes, that unfortunately failed to shepherd $A_3$AR overexpressing cancer cells. However, ongoing structural optimization of this structures opens the door to the investigation of new possible applications of $A_3$AR ligands in cancer treatment.

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OC-28

# THE FIRST *IN VIVO* PROOF-OF-CONCEPT FOR THE EFFICACY OF SELECTIVE HDAC6 INHIBITION IN CYSTIC FIBROSIS: ANTI-INFLAMMATORY PROFILE, EFFECTS ON BACTERIAL LOAD, FORMULATION AND BIODISTRIBUTION STUDIES

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Compelling new support has been provided for histone deacetylase isoform 6 (HDAC6) as a common thread in the generation of the dysregulated proinflammatory phenotype in cystic fibrosis (CF). HDAC6 also plays a crucial role in bacterial clearance or killing as a direct consequence of its effects on CF immune responses. Inhibiting HDAC6 functions thus eventually represents an innovative and effective strategy to tackle multiple aspects of CF-associated lung disease.[1] In this study, we provided the first *in vivo* PoC of the efficacy of a selective HDAC6 inhibitor in contrasting the CF-associated proinflammatory phenotype and effectively reducing bacterial load in treated animals. First, we embarked a careful compound selection by performing a thorough analysis of scientific literature and relevant patents in the field. We thus identified compound **1** as the most suitable candidate to be engaged in *in vivo* PoC studies based on its high HDAC6 inhibitory potency and relevant selectivity over other HDAC isoforms (Fig. 1). We re-profiled compound **1** in house on its HDAC1/6 potency and its ability in selectively increasing levels of acetylated tubulin in HeLa and A549 cells. We also evaluated solubility of **1** in the vehicle selected for the planned aerosol administration. Gratifyingly, compound **1** demonstrated no toxicity in mouse and was able to dose-dependently reduce the total cell counts and neutrophils in bronchoalveolar lavage fluid (BALF), when locally administered (5, 10 and 20 mg/kg) in a mouse model of chronic *Pseudomonas aeruginosa (PA)* infection using a Penn-Century MicroSprayer® Aerosoliser. We also performed a cytokines/chemokines profiling by Bioplex assay, which highlighted relevant changes in the levels of interleukins (eg. Il-1$\alpha$, IL-1$\beta$, IL-6) and other inflammatory markers, thus confirming the potential of **1** in effectively reverting the pro-inflammatory phenotype. Moreover, we proved that **1** is able to reduce bacterial load in the same model at the three tested doses, as determined by the reduction of colony-forming units (Fig. 1). Quantitative determination of **1** in plasma samples collected from treated mice showed that the compound is not distributed in the body even after a 7-day treatment, thus supporting the safe profile of **1** in our administration settings. Our study is of particular significance since it demonstrates for the first time the utility of selective HDAC6 inhibitors as innovative therapeutic option for CF, using a relevant *in vivo* model. Our data pave the way to the development of novel HDAC6 inhibitors specifically tailored for chronic administration in CF patients, thus improving the CF-associated inflammatory phenotype and promoting an effective immune response against infections.

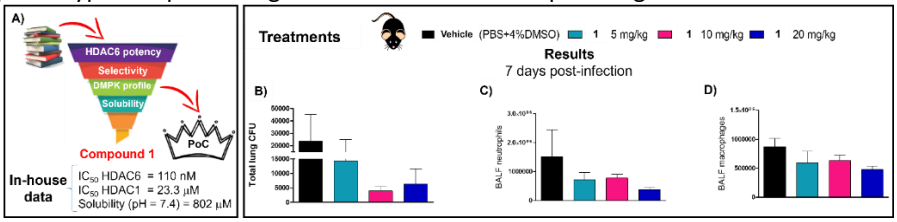


**Figure 1.** (A) General workflow for proof-of-concept (PoC) compound identification; efficacy of selected compound **1** on (A) bacterial load and (B,C) inflammatory cell count in a murine model of *Pseudomonas Aeruginosa* chronic infection.

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OC-29

# NEW INSIGHTS IN THE DEVELOPMENT OF CANNABINOID RECEPTOR SUBTYPE 2 (CB2R) LIGANDS

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Cannabinoid receptor subtype 2 (CB2R) and the cannabinoid receptor subtype 1 (CB1R), belong to the EndoCannabinoid System (ECS), today considered part of a more complex system, i.e. the endocannabinoidome (eCBome),[1] exerting pivotal impact in several diseases.[1,2]

In the last years, CB2R gained a great attention as its activation may mitigate neuroinflammatory events without the psychotropic effects due to CB1R stimulation. Differently from the CB1R, mainly localized in the CNS, CB2R, that is physiologically expressed in the immune system, is inducible in pathological inflammatory states, both in periphery and in the CNS. Diverse classes of ligands have been developed as CB2R agonists and studied for their therapeutic potential in the oncologic and neurodegenerative fields.[2] CB2R agonists are also under investigation for their potential application to face the cytokines storm in Covid-19. Thanks to the recently released crystal structures of the CB2R[3,4] and with the aid of molecular modelling simulation, we designed and synthesized diverse CB2R ligands endowed with high CB2R affinity and selectivity. We also deepened the molecular determinants responsible for the agonists versus antagonists' functional activity. The well-established role of this subtype in many multifactorial pathologies, such as cancer and neurodegenerative disease, makes CB2R an ideal hit for a multitarget strategy:[5] a PCA analysis proposed HDAC, FAAH and sigma receptors as the partner targets for the development of such dual drugs. Thus, we successfully synthesized and evaluated dual drugs acting on CB2R and FAAH (both involved in the ECS system and overexpressed in inflammation states) with promising results.

Moreover, with the aim to better study the mechanisms involving CB2R in inflammatory diseases we developed fluorescent ligands starting from a quinolone scaffold, as CB2R pharmacophore, linked to different fluorescent moieties.[6] Among these ligands, our derivative SM15 emerged for the valuable pharmacodynamic and pharmacokinetic profile that candidates it as a greener and safer tool for CB2R biological evaluation.

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OC-30

# OPTIMIZING THE CHOICE OF 3D QUERY STRUCTURES IN LIGAND-BASED VIRTUAL SCREENINGS WITH PHARMSCREEN

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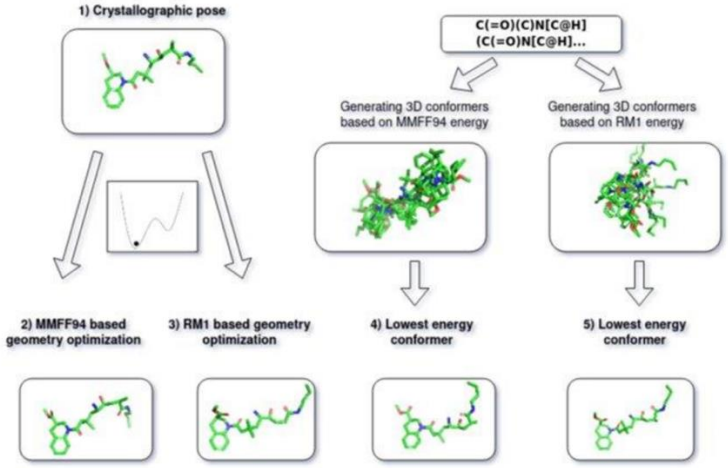
Molecular alignment is a key step in 3D ligand-based virtual screening (3D-LBVS) methods. To perform this step a finite ensemble of conformers per molecule is typically used and aligned to a query molecule. In many projects it is not clear however how to select the query molecule/s and which conformer/s should be used.

In this work, we evaluate various protocols for preparing the 3D structure of the query and assess what is the impact of using a co-crystallized structure vs a minimized structure or using multiple query molecules in 3D-LBVS studies. This evaluation has been done with the 3D-LBVS tool PharmScreen, which exploits the MST-continuous solvent model to define 3D hydrophobic surfaces [1,2] and the DUD-E+ [3] dataset that contains up to 92 pharmaceutically relevant targets and multiple co-crystallized bioactive ligands to each target.




Results show that when using a single reference structure, there are no significative differences between using a low energy conformation and the bioactive conformation on VS performance, in line with previous studies [4,5]. This demonstrates that the 3D bioactive conformation is not essential when performing 3D virtual screenings, allowing purely ligand-based approaches while considering 3D information to search for new bioactive scaffolds. The usage of multiple reference structures, either multiple conformers or multiple active scaffolds shows to be valuable to improve the virtual screening performance and, therefore, should be considered when using 3D-LBVS tools.

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# A COMPUTATIONAL GRID-BASED ANALYSIS TO MAP DRUG-LIKE PEPTIDE BINDING POCKETS OF PEPTIDE-PROTEIN INTERACTIONS SYSTEMS

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Peptide-protein interactions (PePIs) systems play a pivotal role for many cellular and metabolic processes involved in the onset of largely spread diseases such as cancer and neurodegenerative pathologies. Although recent studies have been conducted to get insight into PePIs systems, the identification and characterization of peptide-protein binding sites is still an open challenge.
In this scenario, we investigated physicochemical property space at peptide-protein interface considering an *in house* non-redundant collection of high-quality 3D crystallographic structures of peptide-protein complexes (i.e., PixelDB database[1]). Several interpretable geometrical and energetical 3D GRID-MIFs molecular descriptors have been thus reciprocally computed in order to *in silico* explore the peptide-protein chemical space. Additionally, an exhaustive investigation of 3D-GRID energetic distribution of the most frequent peptide-protein residue pairs at the binding interface has been carried out in order to study the peptide affinity-enhancing interactions compared to the protein-protein systems.[2]
Subsequentially, an innovative machine learning based model has been developed demonstrating to be highly predictive in detecting the putative protein binding regions of small peptides. Based on 3D GRID-MIF molecular descriptors, advanced computational strategies (i.e., clustering algorithm, LDA-based protocol implemented in BioGPS[3]) have been employed to recognize the actual interacting druggable peptide regions at peptide-protein interface. Our model has been successfully challenged on two high-quality external benchmarks of peptide-protein pockets to be effective to further run peptide-protein virtual screening campaigns.
We are confident that our encouraging results pave the way to get insights the identification and structural-energetic characterization of drug-like peptide binding pockets to be further exploited in drug design strategies.

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# NOVEL CYCLIC UPA-DERIVED DECAPEPTIDES REDUCE *IN VIVO* LUNG DISSEMINATION AND RE-EDUCATE CAF PHENOTYPE BY ACTING THROUGH INTEGRIN αvβ5

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In the past, solid tumors were regarded as relatively homogeneous groups of hyperproliferating cells with the ability to invade neighboring tissues and, possibly, metastasize. More recently, a large body of evidence has convincingly revealed that tumors are complex organs composed of multiple cell types and extracellular matrix (ECM).[1,2] Intensive studies on solid mammary, lung, intestinal and prostatic cancer have described a surrounding tumor microenvironment (TME), that includes cancer-associated fibroblasts (CAFs). Mechanistically, CAFs can contribute to cancer progression via integrin-linked mechanisms, through the generation of pro-migratory tracks favoring cancer cell invasion in the stromal ECM.[3,4] This communication is focused on the design, conformational and functional analysis of novel decapeptides endowed with the ability to prevent tumor migration and invasion. Previous work indicates that the serine protease urokinase (uPA) has a catalytically-independent motogen activity that resides in its connecting peptide region (CP, residues 132–158).[5,6] Most of the chemotactic activity of the CPp (residues 135-158) is retained by its C-terminal segment uPA-(144–158). In contrast, the N-terminal, uPA-(135–143) peptide, is a potent cell migration inhibitor.[7] Conformational analysis of the CP-derived, anti-migratory peptides, have suggested the development of novel linear and cyclic peptides with specific substitutions. The novel decapeptides inhibited migration and invasion of HT1080 fibrosarcoma and MDA-MB-231 breast carcinoma cells without affecting cell proliferation and apoptosis. They also induced a partial reversion of the CAF phenotype and markedly reduced the pro-invasive ability of peritumoral CAFs from breast cancer patients in combination with MDA-MB-231 mammary adenocarcinoma cells.[8] Lead cyclic peptide, named uPAcyclin, prevented mouse lung metastases by HT1080 cells *in vivo* and inhibited vasculogenic mimicry formation by glioblastoma cells. Regarding the mechanism of action, uPAcyclin is a nanomolar ligand selective for αvβ5 ($K_d$ 7.5 nM) and is unable to compete with vitronectin for binding to αvβ5 in an ELISA competitive assay.
Therefore, uPAcyclin is likely to interact with high affinity and specificity to αvβ5 integrin via an RGD-independent site, suggesting its further development as a novel anti-invasive agent.

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# SCREENING OF AMINO-ACID-ANTHRAQUINONE CLICK CHEMISTRY CONJUGATES TARGETING HUMAN TELOMERIC G-QUADRUPLEXES

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Nucleic acids are flexible biomolecules that can fold into peculiar three-dimensional arrangements. Besides the canonical double strand, sequences showing repeats of guanines (Gs) can form secondary structures known as G-quadruplex (G4), for which multiple roles in gene regulation are well supported. Most importantly, such G-rich sequences that can give rise to the peculiar G4 arrangement have been detected in human oncogenes and in the telomeric regions of chromosomes.[1]

This aspect is of primary relevance in medicinal chemistry, since shortening of telomeres during replication leads to cell senescence and limits its lifetime but telomerase enzyme, inactive in somatic cells, is reactivated in 90% of cancer cells in which it elongates outer ends of chromosomes, leading to immortalization. Interestingly, G4s constitute an obstacle to functioning of telomerase, and G4 stabilization using small molecules therefore represents an appealing strategy for developing novel therapeutic agents.[2,3]

G4 ligands quarfloxin and CX-5461 reached clinical trials (ClinicalTrials.gov IDs: NCT00780663 and NCT02719977), but no GQ ligand has been approved yet due to lack of bioavailability, toxicity issues and overall insufficient drug-likeness. On the other hand, ligands based on an anthraquinone scaffold and decorated with peptidic side chains were previously reported.

In this contribution, we describe the improvement of this ligand concept leveraging the click chemistry approach. This flexible, high yielding synthetic strategy allows an elongation of the side chains and an increase of π–π stacking and H-bond interactions with the nucleobases through the triazole ring.[4]

The synthesized compounds were tested for their ability to interact with G4 DNA with a multiple analytical approach, demonstrating a selectivity for G4 over double stranded DNA. In particular, we report the setup and application of an assay based on the use of native electrospray-mass spectrometry (ESI-MS) to quantitatively detect non-covalent interactions between small molecules and G4s.

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# FLASH COMMUNICATIONS

FC-01

# MACHINE LEARNING APPLIED TO EARLY PREDICTION OF DRUG METABOLISM

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Metabolic reactions impact on determining drug fate: endogenous reactions e.g., (de)activation and (de)toxification have a crucial role in the modification of a drug[1]. Moreover, metabolism accounts for roughly the 70% of drug clearance[2]. For these reasons, drug metabolism represents a key phase in drug screening for drug discovery. Machine Learning (ML) can be applied for the prediction of drug metabolism, to allow identifying the interaction between drug candidates and endogenous metabolic reactions, drug metabolizing enzymes (DMEs) driven reactions, biotransformation pathways, the generation of potential toxic metabolites or ADMET properties. ML tools include the application of in silico models e.g., quantitative structure-activity relationship (QSAR) based on trainable database, which represent the leading "fail early and fail cheap strategy"[1].

Exploiting the Machine Learning approaches, ACD/Percepta and ChemTunes/ToxGPS software can be fruitfully employed to predict early drug metabolism. ACD/Percepta gives the opportunity to predict cytochrome P450 Inhibition, P450 Substrates specificity and Regioselectivity of Metabolism, covering five major isoforms (i.e., CYP3A4, CYP2D6, CYP1A2, CYP2C9 and CYP2C19)[3]. The quality and reliability of the prediction can be assessed by means of a Reliability Index (RI). ACD/Percepta models have been developed using wide datasets, providing robustness to the model and to each prediction, which is also supported by experimental data from the training set. ACD/Percepta models present the advantage of being trainable with additional experimental data provided by the users. ChemTunes/ToxGPS Liver BioPath allows to generate metabolites of a molecules, deriving from human phase I and phase II reactions[4].

The scopes of this communication are to present how early drug metabolism can be predicted by ML algorithms, to discuss the prediction quality and reliability, and to underline how this proves to be a cost-effective approach.

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# EXTRA VIRGIN OLIVE OIL EXTRACTS ENRICHED IN SECOIRIDOIDS INDUCE AN ANTI-INFLAMMATORY PROFILE IN PBMCs FROM OBESE CHILDREN

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Extra virgin olive oil (EVOO) is one of the most important functional foods from the Mediterranean Diet and exerts health-promoting effect for different pathological conditions not only due to its major (monounsaturated fatty acids) but also to its minor (phenolics) components, as reported in the last years[1]. Several studies demonstrated the EVOO anti-inflammatory activity to reduce inflammation, a typical feature of chronic disorders[2]. Among these, obesity represents an important public health challenge of the 21st century reaching epidemic proportion worldwide. In particular, more than a tenfold increase in the number of school-age and adolescents with obesity in the last four decades was reported[3].
Therefore, starting from our in vitro data identifying a defined EVOO chemical profile correlating with an anti-inflammatory effect[4], we studied the ability of this secoiridoids-enriched EVOO extract to modulate the inflammatory profile ex vivo. Specifically, peripheral blood mononuclear cells (PBMCs) collected from obese children were treated with secoiridoids-enriched EVOO and refined olive oil (OO) extracts, characterized by a low polyphenol content, to study the ability of secoiridoids to dampen the inflammatory response. A specific reduction of proinflammatory $CD14^{+}CD16^{+}$ monocytes was detected by cytofluorimetric analysis when PBMCs were treated with EVOO as compared to OO extracts. According to this, a down-modulation of MIP-1β and MCP-1 chemokines involving in the recruitment of inflammatory cells, such as monocytes and macrophages, was reported at protein level in the surnatant of EVOO relative to OO extracts treated PBMCs. Moreover, the hierarchical clustering on real-time PCR gene expression data studying the PBMCs molecular profile in the contest of the inflammatory pathways, showed a distinct segregation between EVOO and OO when compared to untreated sample. Ingenuity Pathway Analysis (IPA) revealed that the modulated genes were linked to obesity and, importantly, some of these genes were involved in the pathway promoting the development of severe obesity.
Overall, our ex vivo data demonstrated the ability of secoiridoids-enriched EVOO to reduce the inflammatory milieu of PBMCs from obese children at protein and molecular levels. These results could support the use of secoiridoids-enriched EVOO in preventive and/or adjuvant treatments for obesity and severe obesity and, potentially, for the related noncommunicable diseases even if additional studies are needed to verify the anti-inflammatory ability in this pathological context.

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FC-03

# COMBINING MASS SPECTROMETRY AND NUCLEAR MAGNETIC RESONANCE FOR THE STUDY OF LIGAND:G-QUADRUPLEX INTERACTION

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Nucleic acids fold in solution into secondary structures that result in peculiar three-dimensional arrangements. It has been demonstrated that in human, bacterial and viral genome also non-*Watson-Crick* pairings are present, leading to the formation of alternative secondary structures, such as G-quadruplex (G4).[1] Bioinformatic studies highlighted its prevalence at gene regulatory regions, in telomeres, chromatin DNA and in specific RNA sequences.[3] Since enzymatic machineries that process DNA or RNA are hindered by G4s, their stabilization with small molecules represents a strategy for interfering with key cellular functions, such as transcription, translation and telomerase activity inhibiting their unwinding to other nucleic acid species[4] and has been studied for its potential therapeutic effect, especially in uncontrolled cellular proliferation and cancer progression.[5]

Our group tested a series of ligands with different scaffolds, comprehending newly synthetized and natural or semi-synthetic compounds. Our screening protocol includes an optimized *in vitro* and *in silico* screenings based on Electrospray Ionization – Mass Spectrometry (ESI-MS) and molecular docking, respectively. Thanks to ESI-MS technique, which guarantees the conservation of non-covalent ligand-DNA interactions, we can calculate parameters such as the binding affinity. By screening the compounds towards different DNA arrangements, it is possible to investigate sequence selectivity and through collision-induced dissociation (CID) experiments the stability of the complex ($E_{COM}^{50\%}$) can be determined. Molecular docking simulation on the different G4 topologies allows to predict the possible three-dimensional arrangements upon binding. The screening of the same compounds through NMR techniques is currently ongoing, with the collaboration of Goethe University where I am currently attending the research project, performing 1D NMR titrations and 2D HSQC, NOESY, HMBC on the sequences with the most promising compounds which would lead us to structural elucidation.

Thanks to the screening, we can obtain a preliminary structure-activity relationship studies. Moreover, the effects of derivatization of natural compounds are being currently explored together with the investigation of the role of alkylating functional groups that can promote covalent DNA modification, allowing an additional mechanism of action for delivering DNA damage. [6-9]

The results of the study of G4-ligands interaction are strongly dependent on the experimental technique and conditions used, therefore the combination of different techniques can give complementary information on structure recognition and binding. ESI-MS has the great advantage to be a rapid screening technique with low sample consumption and NMR gives the possibility to follow each signal and therefore to see real time which parts of the arrangement are involved in the binding.

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FC-04

# CARBAZOLE DERIVATIVES AS MULTI-TARGET AGENTS IN BREAST CANCER TREATMENT

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Over the years, carbazoles are largely studied for their numerous biological properties, including antibacterial, antimalarial, antioxidant, antidiabetic, neuroprotective, anticancer and many more.[1] In particular, this scaffold has gained great interest due to its anticancer activity on breast cancer, which still represents the most frequently diagnosed cancer in women and remains a serious health emergency despite many research efforts.[2] Considering the complexity of this disease, the toxicity of traditional therapeutic treatments and the resistance onset risk, it is urgent the development of new and effective anti-breast cancer agents with improved pharmaceutical profiles. In this context, we studied the anticancer activity of a series of carbazole compounds[3] (Figure 1) against two breast cancer cells, namely MDA-MB-231 and MCF-7 cells. Among them, compounds **3** and **4** were found to be the most active towards the triple negative breast cancer cell line MDA-MB-231, which is the most aggressive and metastatic ($IC_{50}$ values equal to 1.44±0.97 and 0.73±0.74 µM, respectively). Furthermore, they did not interfere with the growth of the normal cell line MCF-10A, contrarily to Ellipticine, a well-known carbazole derivative, used as a reference molecule. Both compounds selectively inhibited the human topoisomerase I and were also able to interfere with the regular organization of the actin system, triggering apoptosis as final effect. Thus, compounds **3** and **4** could represent promising candidates to be further investigated for the development of new multi-target agents in the treatment of triple negative breast cancer, for which safe therapeutic regimens have not yet been adopted.

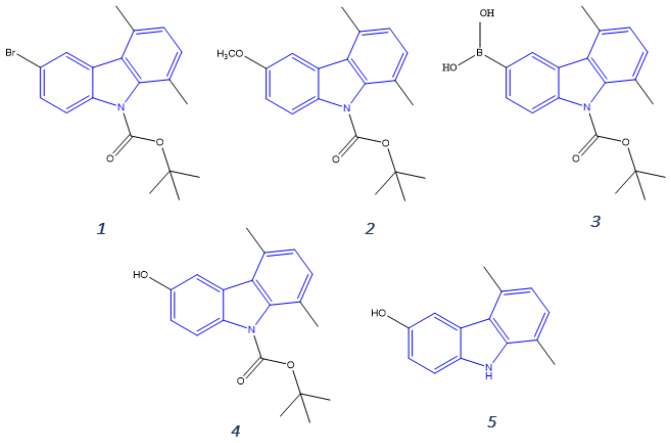


**Figure 1.** Molecular structures of the studied carbazole derivatives (**1**-**5**).

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FC-05

# TARGETING THE MYCOBACTIN BIOSYNTHESIS PATHWAY IN *M. TUBERCULOSIS*: A STEP TOWARDS THE IMPROVEMENT OF THE ANTI-VIRULENCE ACTIVITY OF MbtI INHIBITORS

Mori, M.;[a] Villa, S.;[a] Chiarelli, L. R.;[b] Stelitano, G.;[b] Gelain, A.;[a] Cazzaniga, G.;[a] Bellinzoni, M.;[c] Rizzello, L.;[a] Scarpa, E.; [a] Griego, A.; [a] Fumagalli, A.; [a] Meneghetti, F.[a]

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The COVID-19 pandemic has left many countries struggling to ensure an adequate medical support to the population. The difficulties of the healthcare systems have had particularly devastating consequences on the diagnosis and treatment of tuberculosis (TB) infections, especially in the poorest communities. Hence, the development of antitubercular agents is vital, now more than ever.[1]
In this context, the design of anti-virulence compounds, *i.e.*, molecules targeting pathways involved in the pathogenesis of *M. tuberculosis* but not essential for its survival, is emerging as a promising strategy. This innovative approach has several advantages, the principal being the prevention of resistance phenomena. Iron uptake constitutes a notable example of such pathways. Its suppression can be achieved by the inhibition of a mycobacterium-specific enzyme, namely the salicylate synthase MbtI, which is involved in the biosynthesis of high-affinity iron chelators.[2]
Starting from a structure-based virtual screening, our group developed a new class of potent furan-based inhibitors of MbtI.[3] Co-crystallization studies led to the definition of their unexpected binding mode and, incidentally, shed light on the catalytical mechanism of the enzyme.[4] Considering the modest, albeit promising, antimycobacterial effect of the lead compound, we explored the possibility of introducing modifications on its scaffold to enhance the crossing of the mycobacterial cell wall. Inspired by the crystallographic data, we designed new derivatives bearing different lipophilic moieties to increase their permeability, without disrupting the interaction with the enzyme. Based on our encouraging results, the best compounds were tested on infected macrophages to verify their protective effect against the pathogen. The preliminary results of our tests will be presented to support the potentialities of this novel antitubercular strategy.

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FC-o6

# DEVELOPMENT OF HYDROGEN SULFIDE-RELEASING HYBRIDS AS NOVEL MULTITARGET DRUGS

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Hydrogen sulfide ($H_2S$) is now well recognized as the third endogenous signaling gasotransmitter, along with nitric oxide (NO) and carbon monoxide (CO). It is largely known as a pleiotropic mediator endowed with antioxidant, anti-inflammatory, pro-autophagic, and neuroprotective properties.[1] Noteworthy, work over the last decade revealed the importance of $H_2S$ in inflammatory diseases.[2] Moreover, current data suggest the therapeutic potential of $H_2S$ releasing compounds, individually or in combination with other drugs.[3] Among anti-inflammatory molecules, glucocorticoids (GC) are one of the most prescribed. Despite being the most effective anti-inflammatory treatment for chronic inflammatory diseases, some patients seem to be resistant to GC treatment. Hence, aiming to overcome these limitations, we designed and synthesized novel multitarget molecules, combining glucocorticoids with well-known $H_2S$-donor moieties.[4,5] Firstly, synthesized compounds have been evaluated for their potential $H_2S$-releasing profile. Furthermore, the $H_2S$ donor hybrids have been studied in *in vivo* models of inflammation, showing an improved pharmacological activity, simultaneously modulating multiple targets of the native drugs. The most interesting compounds have been selected and further investigated to elucidate the possible mechanism of action. On this basis, the use of $H_2S$-releasing hybrid compounds could represent a potential and intriguing strategy to be pursued for the treatment of inflammatory diseases where $H_2S$ is involved.

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FC-07

# DISCOVERY OF 2-(4-HYDROXY-3,5-DIMETHYLPHENYL)-*N*-(PYRIDIN-2-YL)-1H-BENZO[*d*]IMIDAZOLE-6-SULFONAMIDE AS BET INHIBITOR WITH SELECTIVITY FOR THE FIRST BROMODOMAIN

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Bromodomains (BRDs) are epigenetic readers able to selectively recognize the acetyl-lysine group on histone and non-histone proteins. The Bromo and Extra-Terminal Domain (BET) family is perhaps the most important group of BRD-containing proteins, whose members (BRD2, BRD3, BRD4, and BRDT) contain two highly homologous bromodomains: BD1 and BD2.[1] Their role in different biological processes makes them attractive therapeutic targets.[2] To date, several ligands have been identified as potent BET ligands and some of them have been entered in clinical trials.[3] However, most of these compounds are pan BET ligands, with no selectivity between each BET member and/or their singular BD1/BD2 domains.[4] Thus, the identification of selective chemical probes for the clear definition of the physio-pathological role of individual BET domains is highly demanded.
Herein, we report the design, the synthesis, and the in vitro evaluation of a small library of benzimidazole-based compounds (Figure 1). The benzimidazole nucleus was formally obtained applying a frozen analogue approach to the diazobenzene moiety of MS compounds that exhibited preference for the BD1 over the BD2 of BETs.[5] The most promising compound identified, **EML765**, showed good binding potency, improved metabolic stability and selectivity towards BD1 with respect to the parent compounds.

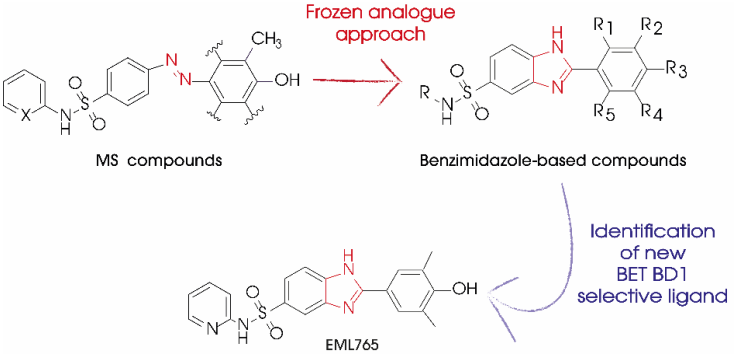


**Figure 1.** Aim of work: Identification of benzimidazole-based compounds as BD1 selective ligands.

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# FIRST-IN-CLASS SELECTIVE INHIBITORS OF THE HISTONE ACETYLTRANSFERASE KAT8

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KAT8 (also called MOF) is a histone acetyltransferase (HAT) mainly catalyzing the acetylation of Lys16 of histone H4 (H4K16), as well as transcription factors such as p53 and Nrf2.[1] KAT8 is the catalytic subunit of the male-specific lethal (MSL) and the non-specific lethal (NSL) multiprotein complexes, which regulate cell cycle progression, embryonic stem cell development, DNA damage response, autophagy, and apoptosis.[1] Given its manifold functions, KAT8 dysregulation is linked to the onset, progression, and metastasis of multiple cancer types, including non-small cell lung cancer (NSCLC), breast cancer, acute myeloid leukemia (AML), endometrial, and hepatocellular carcinoma.[1] In line with this, disruption of the MSL complex, and consequent loss of the H4K16ac mark, causes chromosomal instability and abolishes the proliferative potential of cancer cells.[2] Hence, potent and selective KAT8 inhibitors (KAT8i) would represent promising anticancer therapeutics, beyond serving as chemical tools to clarify KAT8 function. To date, only a few KAT8i have been reported, and none of them displayed selective activity.[1] To this end, based on the structure of C646, a known p300[3] and HDAC[4] inhibitor, we developed a series of derivatives bearing a phenylpyrazolone core (**1**-**43**, Fig. 1) which were tested for their activity against three HATs: KAT8, p300, and PCAF.

Among them, compounds **19** and **34** exhibited $IC_{50}$ values in the low micromolar range (8-12 µM), along with selectivity over p300 and PCAF, as well as a panel of HDACs. They also possessed dissociation constants ($K_D$) measured *via* surface plasmon resonance (SPR) of 2-4 µM, in line with the observed KAT8 inhibition. Finally, we validated both inhibitors in a panel of cancer cells, including NSCLC (H1299 and A549), breast cancer (MCF7), colorectal carcinoma (HT29 and HCT116) and cervical carcinoma (HeLa). Cell-based assays demonstrated selective decrease of H4K16ac induced by **19** and **34** thereby confirming their ability to inhibit KAT8 in cells. Moreover, both molecules impaired cell viability of NSCLC, colorectal and cervical carcinoma cell lines in the mid-micromolar range, while not affecting the viability of non-transformed cells (AAH1 e RPE) at 200 µM. Overall, through this study, we developed first-in-class selective and cellularly-active KAT8i which represent promising tools for elucidating KAT8 biology. Given their simple structure, these molecules are also encouraging lead compounds for future studies aimed at gaining submicromolar/nanomolar KAT8i.

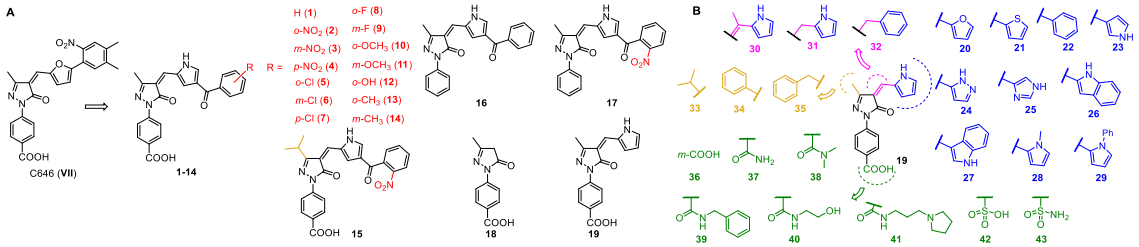


**Figure 1.** (A) Development of compounds **1**-**19** starting from C646 (**VII**). (B) Chemical structures of compounds **20**-**43**.

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FC-09

# DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NEW HYBRID MOR AGONIST/HDACI COMPOUNDS: AN INNOVATIVE APPROACH FOR PERSISTENT PAIN MANAGEMENT

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Inflammatory pain is a pathological condition caused by the inflammatory response to a tissue damage. Despite existing different pharmacological approaches, nowadays, opioid analgesics continue to be the hallmark of acute and chronic pain treatment[1]. Opioid analgesic effects are predominantly mediated by mu opioid receptor (MOR), whose activation elicits also severe adverse effects. Given the impossibility to segregate the analgesic effect from side effects arising from MOR activation, recently the medicinal chemistry approach focused on the new target involved in pain transmission, such as the histone deacetylase (HDAC) enzyme. Several evidences suggested that epigenetic regulation is involved in development and maintenance of chronic pain. It has been experimentally demonstrated that inflammatory conditions increase the expression of HDAC and that histone deacetylase inhibitors (HDACi) attenuate inflammatory pain. Moreover, the hypoacetylation state of histones H3 and H4 contributes to the decreased expression of MOR in the dorsal root ganglion (DRG)[2]. Indeed, HDACi reversed the up-regulation of HDAC enzymes and restored the MOR expression[3]. For these reasons, hybrid MOR agonist/HDACi molecules could represent a good strategy for the management of inflammatory pain. Multitarget MOR agonist/ HDACi compounds have been designed recurring to the "merging" approach[4] and have been synthesized through classical synthetic methods (Figure 1). Intermediates and final compounds have been appropriately purified through flash chromatography. The structural characterization was determined by $^{1}$H-NMR, $^{13}$C-NMR and MS.

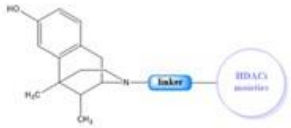


**Figure 1.** General structure of novel hybrid compounds

In *vitro* their affinity profile *versus* opioid receptors was performed through competition binding assays. Some assayed compounds showed a relevant MOR affinity with $K_i$ values in the range of 1.5-5.9 nM and the most promising molecules were tested *ex vivo* by *mouse vas deference* (MVD) assay. The preclinical evaluation of the new ligands is currently ongoing. Due to the encouraging results, a novel compound is undergoing in *in vivo* studies to evaluate its pain-relieving activity in Tail flick assay and in complete Freund's adjuvant (CFA) inflammatory pain model. Moreover, the HDAC fluorometric assay is programmed to study the inhibition properties of our new synthesized compounds.

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# VISIBLE-LIGHT PHOTOCATALYTIC ACTIVITY OF ISOCYANIDES: FROM THE PROOF-OF-CONCEPT TO THE SYNTHETIC APPLICATION IN *UGI-LIKE* CHEMISTRY

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Isocyanides represent a class of very special organic compounds, thanks to their chameleonic electronic properties and multiple reactivity modes, spanning from carbene/nucleophile, to electrophile, to somophile behaviors. Their ability to act as geminal radical acceptors has recently been undergoing a renewed interest, with the advent of the visible-light photocatalysis era. Whitin this context, herein we present our recent findings about the photocatalytic activity of both aromatic and aliphatic isocyanides in the α-amino C($sp^3$)–H functionalization[1] (Figure 1). While aromatic isocyanides can reach an electronically excited state upon visible-light absorption, and thus promote the oxidation of aromatic tertiary amines, aliphatic isocyanides are able to induce the oxidation of tertiary aromatic amines *via* the formation of an electron-donor-acceptor (EDA) complex. These photocatalytic properties can be harnessed in the three-component cross-dehydrogenative coupling of *N,N*-dimethylaniline derivatives with isocyanides and water, leading to amide products under very mild conditions, in high yields, and with a wide substrate scope. Moreover, the use of a catalytic loading of an aromatic isocyanide promotes the cross-dehydrogenative coupling of *N*-phenyl-1,2,3,4-tetrahydroisoquinoline with a range of (pro)nucleophiles affording Mannich, Strecker, *aza*-Henry, Michael-addition, and phosphonylated adducts in good to excellent yields, thus providing the proof-of-concept for their use as a new class of organic visible-light photocatalysts. Furthermore, the photocatalytic activity of isocyanides can be preserved in the presence of different nucleophiles to perform visible-light triggered *Ugi-like* multicomponent reactions under unprecedented metal-free reaction conditions[2]. A collection of different transformations leading to multifunctional molecular architectures has been developed; they include: (1) Ugi-3CR (Nu: both aliphatic and aromatic carboxylic acids), (2) Ugi-tetrazole-3CR (Nu: $TMSN_3$), (3) Jouillè-Ugi-3CR (cyclic tertiary aromatic amines as starting materials), (4) synthesis of secondary imides by using 2,4- dimethoxybenzyl isocyanide as a cleavable one, and (5) a one-pot domino sequence of Ugi-3CR/deprotection/Mumm transacylation affording densely functionalized bis-amide derivatives.

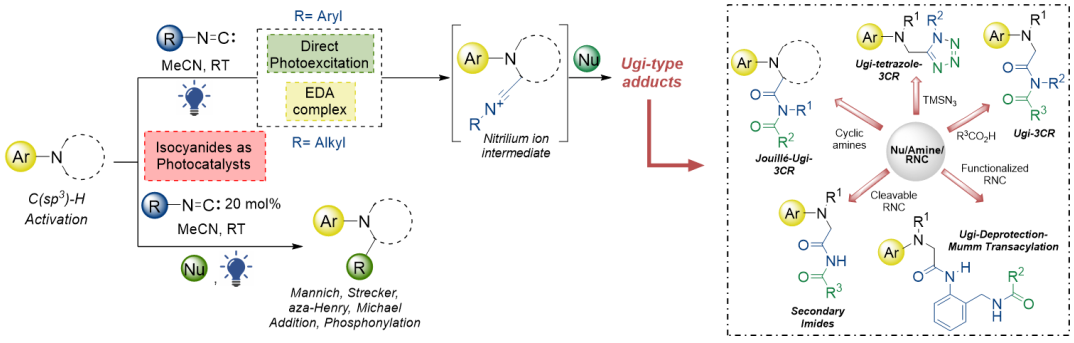


**Figure 1.**

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# TETRAHYDROPYRAN AND CYCLOHEXANE LINKED NOVEL BACTERIAL TOPOISOMERASE INHIBITORS WITH IMPROVED BALANCED ANTIBACTERIAL ACTIVITY AND SAFETY PROFILE

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Bacterial type II topoisomerases, such as DNA gyrase and topoisomerase IV (topoIV) are well-validated targets for antibacterial chemotherapy. Inhibition of their function leads to perturbations in the native spatial DNA topology, which results in bacterial cell death.[1] Two decades ago a new class of promising antibacterials known as '**N**ovel **B**acterial **T**opoisomerase **I**nhibitors' (NBTIs) were discovered.[2] However, until today there is no antibacterial agent from this class on the market. Despite their potent antibacterial activity, they suffer from a detrimental class-related hERG blockage manifested as cardiotoxicity and arrhythmias.[3] We designed and synthesized an optimized library of NBTIs that exhibit improved hERG safety profile and retained inhibitory potencies on DNA gyrase and topoIV from *S. aureus* and *E. coli.* The balance between antibacterial activity and safety (hERG inhibition potency) should be achieved for the compound to be an effective antimicrobial drug. A significant parameter that correlates these two important properties is the hERG/MIC ratio. The higher the ratio, the more optimal safety profile compound exhibit. It is evident that compounds **2** and **3** have 3.5–12 fold higher hERG/MIC ratio compared to their predecessor **1** in Gram-positive bacteria (Figure 1).[4] This suggests that an acceptable therapeutic window can be achieved in Gram-positive bacteria (e.g., *S. aureus*, MRSA). However, for Gram-negative bacteria the hERG/MIC ratio is of particular concern, since the antibacterial activities of the studied NBTIs are notably weaker and nearly in the same order of magnitude. Consequently, a further optimization is needed in terms of achieving an optimal balance between the antibacterial activity and safety profile of this series of NBTIs.[5]

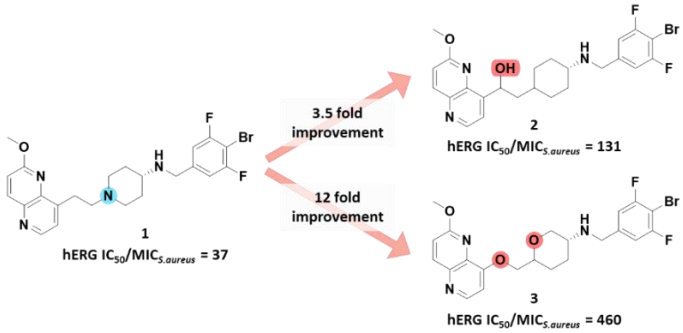


**Figure 1.** Structural optimization strategy towards reducing hERG inhibitory activity of aminopiperidine linked NBTIs with 1-hydroxyethylene cyclohexyl and oxymethylene tetrahydropyranyl linker moiety.

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# MICONAZOLE-LIKE SCAFFOLD IS A PROMISING LEAD FOR DEVELOPING *NAEGLERIA FOWLERI*-SPECIFIC BRAIN PERMEABLE CYP51 INHIBITORS

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Primary amoebic meningoencephalitis (PAM) due to the free-living ameboflagellate *N. fowleri* is a fulminating brain infection that can result in death within days, with a worldwide distribution and over 97% fatality rate.[1,2] Currently, there is no standard regimen for the treatment of *Naegleria* infections in humans. Only seven patients out of 381 reported PAM cases worldwide have been treated successfully with Amphotericin B (AmpB), either alone or in combination with other drugs.[3] However, clinical use of AmpB is limited due to its toxicity, including acute infusion-related reactions and dose-related nephrotoxicity.[4] For these reasons the development of effective and safe drugs for the PAM treatment represents a real unmet medical need. Over the past few years, we validated several steroidogenic enzymes as drug targets. In particular, disruption of CYP51 function by sterol biosynthesis inhibitors, induced massive autophagocytosis in cultured trophozoites leading to *N. fowleri* cell death after 24h of drug exposure.[5] Notaby, *in vitro* growth inhibition of *N. fowleri* by sterol biosynthesis inhibitors, including antifungal azole drugs, has been reported in literature and a variety of FDA-approved CYP51 inhibitors, such as miconazole, have been used in combination therapies with AmpB for the treatment of PAM patients. [6,7] In this work, we provide evidence that miconazole analogs could be considered as drug candidates for the treatment of PAM. We used a combination of cheminformatics, biochemical, X-ray crystallography and phenotypic methods to identify a lead scaffold conducive to blood-brain barrier (BBB) permeability. 124 compounds pre-selected *in silico* with an average MW of 345.6 ± 43.1 and cLogP of 3.79 ± 0.50 were tested against *N. fowleri* trophozoites, allowing to identify nine hits (**1a-i**) with $EC_{50} \leq 10$ µM. The top hit (**2a**) was identified *via* cross-validation in co-crystallization with the *N. fowleri* CYP51 (NfCYP51) target that singled out a miconazole-like scaffold having the best drug-target fit. Based on the co-crystal structure, eleven new analogs were synthesized and biochemically and structurally characterized to assess the optimal configuration of substituents at the phenyl- and benzoyl moieties and the role of the ester linker. Some of the newly synthesized compounds exhibited improved $EC_{50}$ and $K_D$ compared to **2a**, demonstrated better drug-target fit, and indicated brain penetration potential. Synthetic pathways, *in vitro* activities, X-ray crystallography data and pharmacokinetic (PK) study will be shown and discussed.

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FC-13

# STRUCTURAL MODIFICATIONS OF TRIAZINE-BASED COMPOUNDS FOR HIGH-EFFICIENCY PDK INHIBITION

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Deregulation of cellular metabolism has gained increasing interest for its role in sustaining survival, proliferation, migration and invasiveness of malignant cancer cells.[1] Several research efforts have focused on developing novel agents targeting protein kinases or pathways involved in cancer cell metabolism. Particularly, among the main regulators of metabolic imbalance of tumor cells, the pyruvate dehydrogenase complex and pyruvate dehydrogenase kinase (PDK) isoforms 1–4 play a key role in metabolic shift from oxidative phosphorylation to aerobic glycolysis, known as Warburg effect and associated with resistance to standard chemotherapy.[2] Moreover, overexpression of PDK isoforms was observed in several types of cancer and is frequently related to cancer resistance, invasion and metastasis. Besides the dichloroacetate (DCA) and DCA mimetics, characterized by side effects and lack of specificity, few chemotypes of PDK inhibitors have been described, and more specific and selective molecules able to halt the PDK activity are needed.[3] Therefore, in order to discover new PDK inhibitors we previously synthesized a series of 1,2,4 triazine compounds, characterized by an excellent degree of PDK inhibition with a subtype selectivity for PDK1 and PDK4 isoforms, ability to reduce cell migration and induce apoptosis. Moreover, the most promising derivatives induced the depolarization of mitochondrial membrane potential (MMP) of cancer cells with mitochondrial damage and cancer cell death. Considering these promising results, we focus our attention on the structural modifications of these triazine-based compounds, in an effort to provide new insights into how to improve the potency and selectivity against PDK1. Herein we report the synthesis and biological evaluation of the new library of triazine analogues, providing a deep characterization of their ability to inhibit the PDK enzymatic activity, anticancer properties and their role in cancer cell metabolism.

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# IN SILICO ASSISTED DISCOVERY OF DUAL 5-LOX/sHE INHIBITORS: IN VITRO CHARACTERIZATION AND IN VIVO ANTI-INFLAMMATORY PROPERTIES

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The inflammatory response involves several converging signaling pathways that coordinate the level of inflammatory mediators and the recruitment of inflammatory cells.[1] The arachidonic acid (AA) cascade represents a key biochemical route for targeting inflammatory pathological conditions. AA is synthetized from membrane phospholipids by cytosolic phospholipase A2 (cPLA2) and transformed via three separate enzymatic systems: a) COXs lead the conversion into prostaglandins (PG) and thromboxane, b) cytochrome P450 (CYP450) transforms AA to anti-inflammatory epoxyeicosatrienoic acids (EETs), that are converted to the pro-inflammatory dihydroxyeicosatrienoic acids (DiHETrEs) by epoxide hydrolase (sEH) c) lipoxygenases (LOs) drive AA conversion to leukotriens.[2] On these premises the synergic inhibition of 5-LOX and sEH represents a valid approach for the development of a new class of anti-inflammatory drugs. Therefore, the present work describes the design, the synthesis, and the pharmacological characterization of indoline-based 5-LOX/sEH double inhibitors. The development of these molecules was carried out starting from a virtual screening protocol performed using an in-house molecular library. Nine different zileuton-inspired[3] molecules were initially selected. The in vitro analysis of these molecules revealed compound **43** as a suitable, indoline-based hit-compound with low micromolar inhibitory potencies against 5-LOX. Therefore, a structure-based design of compound **43** analogues was carried out, leading to the synthesis of 19 new molecules. These molecules underwent to extensive in vitro testing revealing a remarkable dual inhibitory profile over 5-LOX and sEH, rationalized on the basis of molecular modelling studies. Compound **73** emerged as the most potent compound of the series ($IC_{50s}$= 0.41±0.01 μM and 0.43±0.10 μM against isolated 5-LOX and sEH, respectively). This is why derivative **73** was challenged for its anti-inflammatory activity in two in vivo murine models (zymosan-induced peritonitis and ovoalbumin-induced asthma). Compound **73** displayed remarkable in vivo anti-inflammatory properties, suggesting that 5-LOX/sEH dual inhibitors can be taken into consideration for further development as therapeutic tools in inflammatory diseases.

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# POSTER PRESENTATIONS

PO-001

# TWO MIXED VALENCE DIRUTHENIUM (II,III) ISOMERIC COMPLEXES SHOW DIFFERENT ANTICANCER PROPERTIES

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Platinum-based agents, i.e., cisplatin, carboplatin and oxaliplatin, are some of the most used and active chemotherapeutic drugs currently prescribed for the treatment of many types of human cancer [1]. However, behind their effectiveness, their use is limited by drug resistance and adverse effects[2]. Accordingly, several effective strategies, including the use of transition metals different from platinum, have been developed[3]. Among these, paddlewheel ruthenium-based complexes characterized by the presence of a direct metal-metal bond and a (II,III) mixed valence are very interesting[4]. In this regard, we recently developed the $Ru_2$(II,III) compound $[Ru_2(EB106)_4Cl]$ where EB106 is an indolylglyoxylyl dipeptide endowed with anticancer activity against glioblastoma multiforme (GBM) cells (Figure 1) [5,6]. Altogether, $[Ru_2(EB106)_4Cl]$ -at variance with EB106- was completely inactive in GBM models, as a consequence of its high stability that in turn is due to the nature of the ligand; namely, the steric protection operated by the indolylglyoxylyl moiety prevents the attack of the water molecules or other potential ligands at the Ru metal centers, inhibiting the release of active ligands, and therefore its anticancer effect. Thus, to deepen our studies on these systems, we developed the complex $[Ru_2(EB776)_4Cl]$ (Figure 1), featuring EB776 (isomer of EB106), which coordinates the $Ru_2$ core.[7] This complex should be more reactive -and thus more active as anticancer agent- because of the increased accessibility of the $Ru_2$ core to the attacking nucleophiles, compared to $[Ru_2(EB106)_4Cl]$.

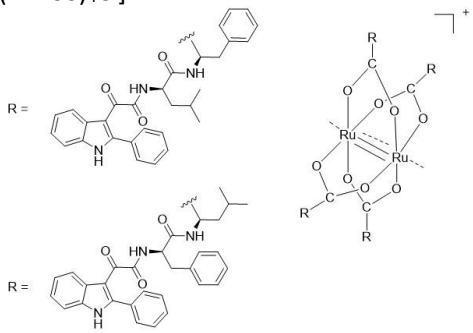


**Figure 1.** Structures of EB106 (top left) and EB776 (bottom left) and general structure of the paddle-wheel $Ru_2$(II,III) complexes (right).

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PO-002

# EFFECT OF PHARMACOLOGICAL INHIBITION OF TRANSGLUTAMINASE ON CARDIAC REMODELING IN HEART FAILURE RATS

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**ABSTRACT**

**Background and Aims**: Cardiac fibrosis is involved in all types of cardiac diseases that progress to heart failure. Cardiac fibrosis is characterized by excessive deposition and crosslinking of collagen which leads to myocardial stiffness and ventricular dysfunction. Transglutaminases are collagen cross-linking enzymes that exert several functions during cardiac remodeling. This research was conducted to examine the protective effects of cystamine on isoproterenol-induced cardiac fibrosis in rats.

**Methods and Results:** A model of cardiac fibrosis was established in rats by daily intraperitoneal injection of isoproterenol (7.5 mg/kg/day) for 7 days, followed by 5 weeks of transglutaminase inhibitor (cystamine) injection. Our data showed that cystamine decreased diastolic and mean arterial pressures, and improved lipid profile along with a decrease in liver enzymes, urea, and creatinine in the isoproterenol rat model. Cystamine inhibited the isoproterenol-induced increase in LV fibrosis and hydroxyproline content. Moreover, cystamine modified the mRNA abundance of some profibrotic markers in the LV and LA of the isoproterenol rat model.

**Conclusions:** TG inhibition by cystamine exerted cardioprotective effects on isoproterenol-induced cardiac fibrosis in rats by modulating the abundance of several profibrotic markers. TG1 and TG2 may represent new therapeutic targets for cardiac fibrosis.

**This abstract has been withdrawn**

**References**

Upon request

PO-003

# A NOVEL CYANINE-BASED NIR FLUORESCENT VEMURAFENIB ANALOG TO PROBE THERAPEUTIC BRAF INHIBITION IN MELANOMA CELLS

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One of the most common skin cancers is cutaneous melanoma, which affects many people per year, ranking 15$^{th}$ among most common cancers worldwide. One of the most frequent genome aberrations in such disease is represented by BRAFV600E mutation, which causes the transition from a valine to a glutamic acid at position 600 within the kinase domain.[1] BRAF protein is a member of the Raf family of serine threonine kinases, which are part of the Ras/RAF/MEK/ERK mitogen activated protein kinase (MAPK) signal transduction cascade that controls cell proliferation and survival. Behind this mutation, the protein remains constitutively activated with an 800-fold increased kinase activity with respect to its wild-type counterpart, leading to uncontrolled proliferation and growth of cells expressing BRAFV600E. In the past decades, a great deal of research has resulted in the development of a targeted therapy thanks to the discovery of B-Raf inhibitors, represented by Vemurafenib, their greatest exponent.[2] Vemurafenib (Vem, PLX4032, Plexxikon/Roche) was discovered as a highly specific BRAFV600E kinase inhibitor with selectivity against melanoma cells and, it is actually clinically approved for the treatment of metastatic and non-resectable BRAFV600E-mutant melanoma.[3]
In this context, with the aim to develop a fluorescent probe to image therapeutic BRAF inhibition, a modifiable derivative of Vemurafenib was synthesized by replacing *p*-chlorophenyl with *p*-aminophenyl ring generating a free $NH_2$ group, which was then conjugated with a selected fluorophore by means of a polyethylene glycol chain. The biological validation evidenced the ability of the new probe, namely Vem-L-Cy5, to penetrate inside cancer cells, specifically bind to its elective target BRAFV600E, and inhibit MEK phosphorylation and cell growth with a potency comparable to that of the native Vem. Taken together, these data highlighted Vem-L-Cy5 as a useful tool to image BRAF inhibition in melanoma cells in order to acquire precious information for the future design of safer and more effective BRAFV600E inhibitors.

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PO-004

# DESIGN, SYNTHESIS MOLECULAR MODELING AND PHARMACOLOGICAL EVALUATION OF 2,7-DIAZASPIRO[4.4]NONANE DERIVATIVES AS NOVEL SIGMA RECEPTORS LIGANDS

Dichiara, M.;[a] Ambrosio, F. A.;[b] Shah, D.;[c] Gonzàles-Cano, R.,[d]; Coricello, A.;[b] Costa, G.;[b] Lee, S. M.;[c] Son, K. N.;[c] Pasquinucci, L.;[a] Marrazzo, A.;[a] Aakalu, V. K.;[c] Cobos, E. J.;[d] Alcaro, S.;[b] and Amata, E.[a]

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Sigma receptors (SRs) represent a unique receptor class involved in several biological and pathological conditions. Two subtypes are distinguished and termed sigma-1 receptor (S1R) and sigma-2 receptor (S2R), having different structure, biological functions, and pharmacological profile. The S1R is highly expressed in both central and peripheral nervous system, in areas of great relevance for neuroprotection, neuroinflammation, neurotransmission, and neuroplasticity. Alterations in the function of S1R have been associated with neurodegenerative diseases, pain, stroke, and retinal degeneration.[1]
Over the years, spirocyclic compounds have gained increasing interest in the development of bioactive compounds and contribute to a variety of approved drugs and drug candidates.[2] The introduction of a spirocyclic moiety in a molecule grant a peculiar spatial arrangement that may influence important parameters, such as potency, selectivity and physicochemical properties.
Here we report the development of novel 2,7-diazaspiro[4.4]nonane derivatives where the central core has been flanked with hydrophobic groups at a certain distance to the central basic amine being this a common structural requirement of potent SR ligands as identified by previous works (Figure 1).

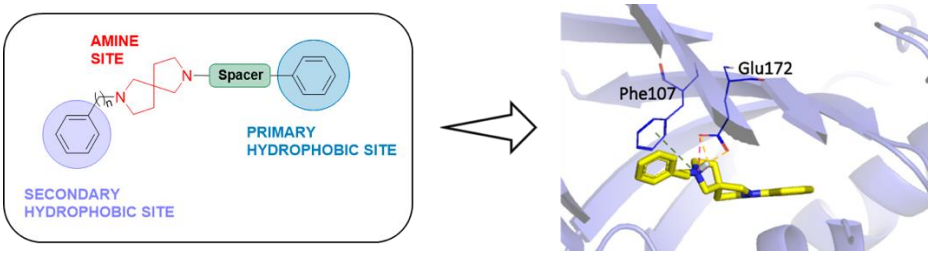


**Figure 1.** General structure of 2,7-diazaspiro[4.4]nonane SR ligands.

This chemical matter has been developed according to structure-affinity relationships approach consisting in the following steps: (i) design of new candidate ligands; (ii) in vitro radioligand binding assays; (iii) iterative compounds design based on affinity and selectivity; (iv) synthesis of the new compounds for further pharmacological evaluation. Several compounds have been synthetized and detailed mechanistic studies performed to understand the binding with the SR. Molecular modeling analysis was carried out to deeply analyze the binding mode and the interactions established between the ligands and S1R and S2R. Finally, the most notable compounds have been subjected to toxicity evaluation and thus screened for phenotypic effects in in vitro and in vivo models.

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PO-005

# DESIGN AND SYNTHESIS OF NEW PEPTIDOMIMETIC MEMBRANOLYTIC COMPOUNDS AND PRELIMINARY EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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Over the years many antibiotics with different mechanisms of action have been developed however most of them undergo to antimicrobial resistance, especially when their mechanism of action depends on enzymatic inhibition.
Nowadays it is known that a non-specific mechanism, such as the membrane physical disruption is less susceptible to resistance, and it could be obtained through specific molecule including antimicrobial peptides (AMPs). Despite their peculiar mechanism of action, there are many reasons that limit the AMPs antimicrobial activity, primarily their high susceptibility to proteolytic degradation by endogenous or microbial enzymes.
To counteract proteolytic degradation and to improve the knowledge around these novel antibacterial, we planned to synthetize a library of antimicrobial peptides overcoming specific weaknesses related to this class of molecules, such as their metabolic instability. Additionally, we attempt to obtain compounds that are not just peptidomimetics but also membranolytic, such as the AMPs, to reach the direct disruption of the bacterial membrane.
The molecular template for the development of our library of peptidomimetics compounds is represented by Brilacidin which is a defensin-mimetic molecule. This molecule was shown to have activity against S. aureus, Enterococcus faecium, E. Coli and SARS-CoV-2.
Based on Brilacidin structure and literature, we identified the hypothetical pharmacophore for the synthesis of structurally related analogues, where side arms length should be modified to obtain the specific conformation for the perpendicular insertion on the phospholipidic bilayers. The firsts compounds have been already synthetized and they show preliminary encouraging antimicrobials activity.

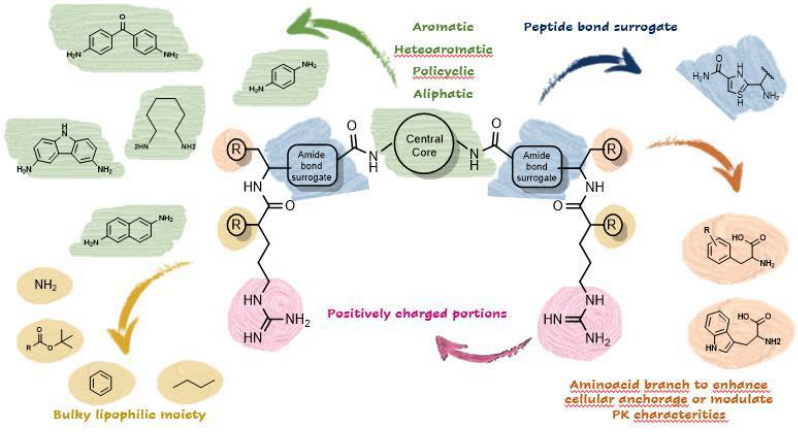


**Fig. 1:** Hypothetical pharmacophore for the synthesis of structurally related analogues

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PO-oo6

# IDENTIFICATION OF COMPOUNDS TARGETING HuD. ANOTHER BRICK IN THE WALL OF NEURODEGENERATIVE DISEASE TREATMENT

Ambrosio, F.A.;[a] Coricello, A.;[b] Costa, G.;[b,c,d] Lupia, A.;[c,d] Micaelli, M.;[e] Marchesi, N.;[f] Sala, F.;[f,g] Pascale, A.;[f] Rossi, D.;[f] Vasile, F.;[g] Alcaro, S.;[b,c,d] Collina, S.[f]

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RNA-binding proteins (RBPs) play a prominent role in the fate of target messenger RNAs (mRNAs). Among the RBPs, HuD (Figure 1) has been intensively studied, it is expressed in nervous tissues, and implicated in the pathogenesis of Alzheimer's disease (AD). The aim of this work is the identification of new ligands able to bind HuD, interfering with its activity. Virtual screening studies were performed using a database of natural compounds and Food and Drug Administration (FDA)-approved drugs. Starting from about 51000 compounds, 10 promising *hits* were selected. Considering the commercial availability and the suitability for NMR analysis, 4 compounds were purchased and submitted to saturation transfer difference (STD) NMR investigations, which proved their ability to bind HuD. For each compound, molecular dynamics simulations were performed to deeply investigate the behavior of the ligands in HuD binding site. Finally, a clear correlation between modeling outcomes and STD-NMR results, was observed. Among the four identified *hits*, folic acid is the most interesting one, being able to well recognize the HuD binding site. Lastly, cell-based assays were performed, and the biological results support the molecular modeling and STD-NMR data, highlighting the ability of folic acid to recognize and affect HuD expression. These findings could be an important starting point for future treatment of some neurological diseases, in particular for AD.[1]

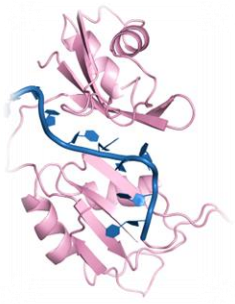

**Figure 1.** 3D representation of HuD-*m*RNA complex. The protein and the mRNA are showed as pink and blue cartoon, respectively.

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PO-007

# HITTING DRUG RESISTANT MALARIA INFECTION WITH TRIAZOLE-LINKED FLAVONOID-CHLOROQUINE HYBRID COMPOUNDS

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Malaria still represents the most severe parasitic infection in humans. Aiming at designing new antimalarials able to circumvent the resistance issues responsible for the failure of first-line therapies, the exploitation of natural-related compounds appears as a promising approach. A small series of hybrid molecules was then designed by linking the 7-chloroquinoline core of the frontline antimalarial drug chloroquine (CQ) to different para-fluorinated chalcone-related scaffolds, in order to take advantage of both the anti-infective activities reported for flavonoids and the efficacy and safety profile of CQ itself[1]. A triazole linker was selected, whose key role as a privileged structural motif in antiparasitic drug discovery has recently been emphasized[2], and compounds (Figure 1) were prepared applying a more sustainable chemistry approach, limiting the use of toxic solvents and operating in mild reaction conditions. The new molecules showed *in vitro* submicromolar antiplasmodial activity against the *P. falciparum* CQ-sensitive D10 strain, and increased potency with respect to chloroquine in the CQ-resistant W2 strain. Derivatives **1b** and **1c** were the most promising compounds of the series, showing the highest *in vitro* activity and a favourable selectivity index with respect to mammalian cells. These compounds also proved to inhibit the host degradation of haemoglobin and the resulting hemozoin formation. Compound **1c** showed a promising transmission-blocking potential, being more effective than CQ against stage V gametocytes of *P. falciparum* parasite; this effect is of pivotal importance for eradicating and preventing malaria infection[3].

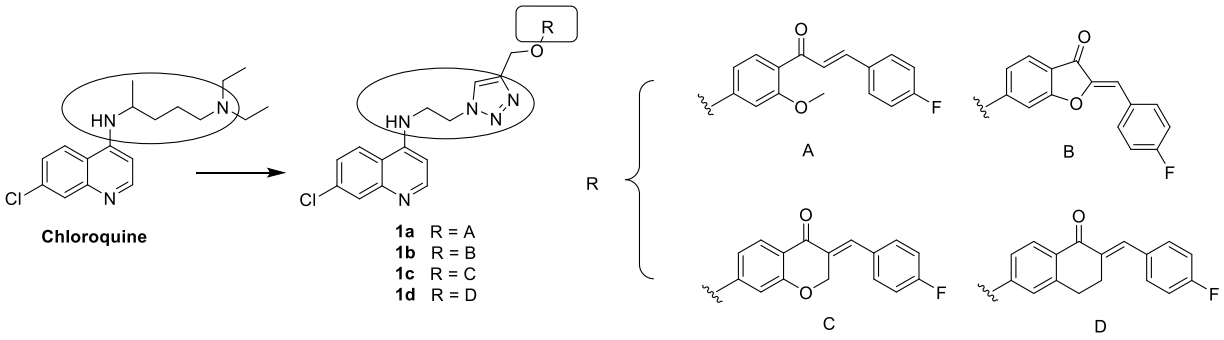


**Figure 1** Design of the new molecules

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PO-oo8

# CARNOSINE DERIVATIVES: THE ROLE OF SECONDARY AMINES IN THE QUENCHING MECHANISM OF REACTIVE CARBONYL SPECIES

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Plenty of investigations have tightly associated carbonyl stress with a multitude of biological mechanisms, like normal age decline as well as different diseases. More in detail, the carbonyl stress pathway is characterized by an increase in the level of reactive carbonyl species (RCS), derived from both endo- and exogenous sources, together with upregulation of the advanced glycoxidation products (AGEs) and its receptor (RAGE)[1]. These RCS interact with the nucleophilic portion of some amino acid residues to form covalent adducts. Moreover, they can also lead to DNA and phospholipids damages. The natural mechanism against carbonyl stress is the detoxification through a combination of aldehyde dehydrogenases and a family of glutathione S-transferase enzymes. Nevertheless, other compounds have the ability to scavenge intracellular RCS, producing unreactive adducts that can be excreted in the urine[2]. One of the most promising pharmacological agents is the natural dipeptide L-carnosine (β-alanyl-L-histidine). This dipeptide has been extensively studied due to its beneficial effects which can involve diverse molecular pathways, including antioxidant, anti-inflammatory, and anti-aggregate properties among others[3]. The RCS-sequestering ability of carnosine is based on a multi-step mechanism that includes Michael addition, Schiff base formation, and/or Paal-Knorr reaction in which both the primary amine and the imidazole ring play a role. Unfortunately, L-carnosine bioavailability is limited due to the rapid degradation by the carnosinase enzyme in tissues and serum[2]. Therefore, L-carnosine analogs endowed with enhanced plasma stability together with efficient RCS scavenging activity need to be developed.

Thus, we synthesized a novel set of L-carnosine derivatives, whose design was inspired by organocatalyst' structures, as well as by the recently published interesting results obtained by L-Pro-L-His [4]. This histidine-containing dipeptide is characterized by a higher scavenging activity together with greater plasma stability than L-carnosine. The stronger affinity toward RSC is due to the secondary amine function that gives an iminium intermediate which in turn is more conducive to the final Michael adduct.

Hence, the role of the secondary amine in the quenching activity has been further investigated by considering different secondary amines, evaluating the role of both the steric hindrance and the electronic properties.

All synthesized compounds have been tested for their quenching activity against 4-hydroxy-trans-2-nonenal (HNE), chosen as prototype of alpha, beta unsaturated RCS produced by lipid peroxidation. The assays comprise monitoring the kinetic by HPLC-UV analysis and the formation of corresponding adducts *via* mass spectrometry analyses.

Finally, the metabolic stability study of the tested compounds in human serum has been also investigated.

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PO-009

# DEVELOPMENT OF PENICILLIN-BASED CARBONIC ANHYDRASE INHIBITOR HYBRIDS FOR THE TREATMENT OF BACTERIAL INFECTIONS

Bonardi, A.;[a,b] Paoletti, N.;[a,b] Gratteri, P.;[b] Capasso C.;[c] Fallani, S.;[d] Cassetta, M.I.;[d] Nocentini, A;[a,b] Supuran, C.T.[a]

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Multidrug resistance against antibiotics currently available in clinics is a main problem of the 21th century. To prevent the risk to enter in a post-antibiotics era, WHO gave some rules in order to repristinate the efficacy of current anti-infective drugs, to avoid the spread of multidrug resistance and to obtain new molecules with an innovative action mechanism.[1] This latter strategy could be achieved targeting new validated antibacterial targets such as the microbial carbonic anhydrases (CA),[2-5] a superfamily of metalloenzymes which catalyzed the reversible hydration of carbon dioxide in bicarbonate and proton.[6] This reaction is important in many physiological processes such as $CO_2$ and $HCO_3^-$ transportation, biosynthetically processes, electrolytic secretion, photosynthesis, pH regulation, virulence, growing and acclimatation of the pathogen.[7] We propose the development of penicillin-based CA inhibitor hybrids to yield new potent antibiotics which combine the innovative anti-infective mechanism based on CA inhibition with that of beta-lactam antibiotics (*e.g.* amoxicillin and ampicillin). The designed hybrids were designed to selectively target bacterial cells according to their affinity for the enzyme D-Ala-D-Ala transpeptidase, important for the synthesis of the microbial wall. The synthetized derivatives were submitted to a wide kinetic enzymatic screening to evaluate their inhibitory activity against CAs from Gram-negative and Gram-positive bacteria and their antibacterial action (MIC) was evaluated against multidrug resistant and ESKAPE bacterial strain.

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PO-010

# NEW PHENOTHIAZINE-DONEPEZIL LIKE HYBRIDS AS POTENTIAL ALZHEIMER'S DISEASE MULTITARGET DRUGS WITH ANTIOXIDANT PROPERTIES

Barbarossa, A.;[a] Leuci, R.;[a] Carrieri, A.;[a] Brunetti, L.;[a] Laghezza A.;[a] Catto, M;[a] Limongelli, F.;[a] Bux, D.; Chaves, S.;[b] Tortorella, P.[a]; Altomare, C.D.[a]; Santos, M.A.;[b] Loiodice, F.[a]; Piemontese, L.[a]; Carocci, A.[a]

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Nowadays it is widely recognized that Alzheimer's disease (AD), a complex neurodegenerative disorder, is the outcome of a wide variety of factors highly interconnected.[1] In the core of such complex scenario, oxidative stress emerges as an important factor in the progression of this disorder since it could trigger many pathological cascades.[2] The limited clinical efficacy of current symptomatic treatment and minute effect on progression of AD has shifted the research focus from single targets towards multitarget directed therapeutic strategies.[3] Thus, the development of multifunctional ligands, possibly endowed with antioxidant capacities, may greatly improve the treatment of the disease. Herein we propose a new family of multitarget directed ligands obtained by linking a phenothiazine nucleus, known for its antioxidant effect, with *N*-benzylpiperidine or *N*-benzylpiperazine fragments, mimicking the core structure of donepezil, one of the few symptomatic drugs approved to date in AD treatment. The investigation of the resulting hybrids (Figure 1) demonstrated, in addition to antioxidant properties, their activity against relevant molecular targets in AD such as the classic cholinesterases (hAChE and hBChE), $A\beta_{40}$ aggregation and the recently proposed endocannabinoid system (FAAH).[4] Furthermore, molecular modelling studies supported the obtained results.

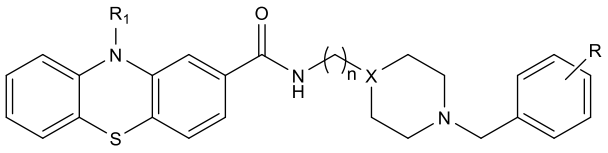


**Figure 1**. General structure for compounds under study.

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PO-011

# SYNTHESIS AND TRYPANOCIDAL ACTIVITY OF NOVEL HYDRAZONES AND THEIR Zn(II) AND Cu(II) COMPLEXES

Cappellacci, L.;[a] Baldassarri, C.;[a] Tombesi, A.;[b] Di Nicola, C.;[b] Pettinari, R.;[a] Marchetti, F.;[b] Verdicchio, F.;[b] Scarpelli, F.;[c] Marangoni, E.;[a] Hofer, A.;[d] and Petrelli, R.[a]

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Human African Trypanosomiasis (HAT), also known as "sleeping sickness", is a neglected tropical disease spread in 36 sub-Saharan African countries. The disease is caused by two protozoan parasites of the genus Trypanosoma, called *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, and is transmitted by the tsetse fly. Despite the recent advances in drug development, few therapies have been approved for the treatment of sleeping sickness, but most of them are toxic, expensive, or ineffective.[1] Therefore, novel drugs with improved safety profiles are needed.
Hydrazones represent a class of compounds endowed with interesting features such as antioxidant, anti-inflammatory, anticancer, antiparasitic, and anti-microbial properties.[2] Recently, pyrazolone-based hydrazones have been used to coordinate Ru(II) affording complexes with promising anticancer activity.[3]
In this study novel pyrazolone-based hydrazones and their Zn(II) and Cu(II) complexes were synthesized and characterized. The biological activity of ligands and their complexes against *T. brucei* was evaluated *in vitro*, as well as the safety profile in mammalian cells.
Moreover, a hypothetical mechanism underneath the anti-trypanosomal activity was proposed.

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PO-012

# METABOLIC PROFILE AND ELUCIDATION OF BIOLOGICAL ACTIVITY OF A STANDARDIZED EXTRACT OF PROCYANIDINS FROM VITIS VINIFERA SEEDS IN HEALTHY VOLUNTEERS

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Proanthocianidins (PACs) from *Vitis vinifera* seeds are a class of bioactive compounds effective in preventing some human diseases such as CVD and diabetes, through an anti-oxidant and anti-inflammatory effect[1]. The molecular mechanism through which these compounds exert their effect is far from clear, especially considering that PACs are not bioavailable as such[2]. The aim of the present investigation is to fully elucidate the main metabolic species in the plasma and urine of human volunteers treated for one week with a dose of standardized extract of PACs (ECOVITIS®)[3] (300 mg twice a day) in respect to a placebo group and to elucidate their biological action. HPLC-MS/MS studies carried out with a triple quadrupole and orbitrap as MS analyzers identified the sulfated and glucurinated forms of 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (γ-V) as the main PAC metabolites after one week of treatment. In particular, after the last dose, the urinary content of the conjugated metabolites, as well as of the free form, increased 4 hours after the last dose reaching a plateau within 12 hours and then decayed time-dependently. The conjugated forms of γ-V arise from γ-V (the main PAC metabolite from the gut microbiota) and our results well indicate that such metabolite is widely absorbed and excreted in conjugated forms in urines. We then explored whether the biological activity of PACs can be attributed, at least in part, to γ-V. For this purpose, γ-V was synthesized, and its biological activity tested in phenotypic cell models to evaluate its anti-oxidant and anti-inflammatory efficacy. The antioxidant activity was tested in a cell model with a reporter gene for Nrf2 activation while the anti-inflammatory activity in cells with a reporter gene for NFκB nuclear translocation induced by TNF-α . γ-V was able to both activate Nrf2 in the range 100-200 µM and to inhibit NFκB activation from a 10 µM concentration. Results also indicate that the molecular mechanism can be attributed to the *orto*-diphenol moiety of γ-V which is activated to the corresponding quinone which is an electrophilic residue able to activate Nrf2 by binding the thiol groups of KEAP1. Nrf2 activation in turn reduces the inflammatory response and hence NFκB translocation by the gene activation of several anti-inflammatory proteins, including heme oxygenase.

To confirm such a mechanism and in particular the activation of anti-oxidant and anti-inflammatory genes related to Nrf2 nuclear translocation, we then isolated the proteome from the buffy coat isolated from volunteers treated with ECOVITIS® and placebo for one week. Quantitative proteomic studies are carried out by using the TMT method and a nano-LC system coupled to a Orbitrap Fusion Tribrid MS analyzer.

In conclusion, we found that PACs are metabolically converted to γ-V which is rapidly absorbed and excreted in conjugated forms such as sulfated and glucuronides. γ-V was found to be a bioactive compound able to induce a Nrf2 induced anti-oxidant and anti-inflammatory activities as found in phenotypic cell models as well as in in vivo conditions by proteomic studies.

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PO-013

# DEVELOPMENT OF A NEW POTENTIAL THERANOSTIC PRODRUG FOR TARGETING GLIOBLASTOMA MULTIFORME

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Glioblastoma Multiforme (GBM) is the most common and highly aggressive primary brain tumor, representing approximately 57% of all gliomas and 48% of all primary malignant CNS cancers. Currently, the standard therapy for GBM includes surgical resection, followed by radiotherapy, and concomitant and adjuvant temozolomide (TMZ). However, GBM remains an incurable disease associated with treatment resistance and high recurrence rates, leading to a poor prognosis with a median survival of 15-16 months and a 5-year survival rate of 5%.[1,2] Therefore, there is an urgent need to develop new effective treatments to fight GBM. Novel therapeutic targets, including kinases, are being explored.[3] Importantly, the aberrant activation of receptor tyrosine kinases (TKs) and their downstream signaling cascade, including the non-receptor TK Src, provide a strong rationale for investigating Src inhibitors in GBM.[4] In recent years, our research group has obtained significant results in developing new compounds active against GBM by inhibiting Src; in particular, the pyrazolo[3,4-*d*]pyrimidine **SI306** and its prodrug **CMP1**, which allowed to overcome **SI306** sub-optimal water solubility, were identified (**Figure 1**).[5] Both compounds were active *in vitro* and *in vivo* GBM models.[5,6] *In vivo*, combination treatment of **SI306** with radiotherapy showed strong activity in arresting tumor growth compared to control and single treatments.[7] Taking advantage of these data, we focused on the development of a new theranostic prodrug (**CMP2**) of **SI306**, bioconjugated with radioactive $^{68}Ga^{3+}$ chelate through a suitable linker. (**Figure 1**). The new agent was designed to target GBM cells after hydrolysis and to monitor the distribution and concentration of the injected labelled molecule through PET imaging. Synthesis of **CMP2** and the first preliminary results will be discussed.

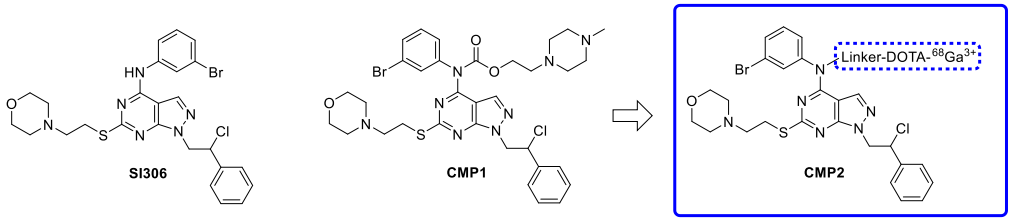


**Figure 1**. Structure of **SI306** and its prodrugs **CMP1** and **CMP2**.

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PO-014

# FUNCTIONAL RESCUE OF F508del-CFTR USING SMALL NITROGEN HETEROCYCLES

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Cystic fibrosis (CF) is a recessive genetic disease found primarily in Caucasians and caused by mutations that impair the function of the CFTR chloride channel. Among the 2000 known CF mutations, deletion of phenylalanine at position 508 (F508del) is the most common one, causing multiple folding and stability defects in CFTR protein which result in premature degradation.[1-3] Pharmacological correction of mutant CFTR defects is an effective therapeutic strategy for CF. In particular, combinations of correctors with complementary mechanisms can be used to maximize the rescue of F508del-CFTR protein.[4]
We have recently identified a very promising class of small molecules, PP compounds, in the rescue of F508del-CFTR in cell lines and in primary airway epithelial cells, particularly in combination with type 1 correctors such as VX-809. Very interesting results emerged for compound PP28 that elicited a strong synergism when combined with VX-809, causing improved trafficking to the plasma membrane and an increased abundance of the mature form (band C) of the protein. A translational approach based on multidisciplinary studies is now driving our efforts to generate more effective and potent analogues as useful tool for precision medicine in CF. Several iterative cycles of chemical synthesis and evaluation of the corrector activity have provided so far useful information about the structure-activity relationship (SAR) of the chemical entities synthetized. More than 300 new compounds have been obtained, clustered into: a) analogues of the parent core; b) new chemical scaffolds. All of them have been tested as correctors i) on primary airway epithelial cells (bronchial and/or nasal); ii) in biochemical assays and by microscopy to evaluate the effect on F508del-CFTR maturation/trafficking. Some new potent analogues emerged as F508del-CFTR correctors, producing a rescue comparable to that of VX-809 and a strong synergism when used in combination with it. The pharmacological insight indicates that PP compounds possibly act as class 3 correctors, since they induce synergistic effect when combined with class 1 (VX-809) and 2 (3151) correctors but not with class 3 (4172). The optimization process of ADME profile is ongoing, in order to improve medchem properties preserving the activity. We aim to obtain the best trade-off between potency/efficacy and "drug-likeness" in order to develop an optimized lead compound that could be considered for preclinical and clinical development.
We acknowledge the Italian Cystic Fibrosis Research Foundation (grant number FFC#3/2020 and Molecules 3.0) and FSE, PON Ricerca e Innovazione 2014-2020 – DM 1062/2021 for fundings. We thank Dr. Tiziano Bandiera, Italian Institute of Technology (IIT), providing correctors 3151 and 4172 for the study.

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PO-015

# NOVEL PYRIDINE-BASED HYDROXAMATES AND 2\'-AMINOANILIDES AS HISTONE DEACETYLASE INHIBITORS: BIOCHEMICAL PROFILE AND ANTICANCER ACTIVITY

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Our research group acquired extensive experience in the design of HDACi in the last two decades.[1-5] In 2001, we described a new class of selective HDACi, 3-(4-aroyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamides (APHAs, **Figure1**, **1**) as agents capable of inhibiting HDAC activity in the micromolar range, characterized by an aroyl moiety as CAP+CU, a pyrrylacrylic moiety as HS, and the hydroxamate group as ZBG.[6] Extensive structure-activity relationship studies performed on the **1** model led to analogues with improved HDAC inhibitory potency and good anticancer properties in mouse A20 cells.[1] Further optimizations on **1** amongst other modifications the replacement of the pyrrole with benzene or pyridine ring in the scaffold led to **2**,[4,5] acting as anticancer agents through HDAC inhibition. A further change of the oxopropenyl with an amide moiety led to the N-hydroxy-3-(acylaminophenyl)acrylamides (**3**)[2] as an additional class of HDACi potent up to nanomolar level.[2] Next we prepared based on compound **3** previously described by us as a HDAC inhibitor, four aza-analogues, as regioisomers containing the pyridine nucleus in various positions. We developed both pyridylacrylic- and nicotinic-based hydroxamates and 2'-aminoanilides to be tested against HDACs. Among them, some nicotinic hydroxamates displayed sub-nanomolar potency and selectivity up to 34 000 times that of HDAC4 and from 100 to 1300 times that of all the other tested HDAC isoforms.[7] The 2'-aminoanilides were class I-selective HDAC inhibitors, generally more potent against HDAC3. The biochemically most potent compounds were tested in several cancer cell lines such as in K562, HCT116, and A549 cancer cells, displaying antiproliferative $IC_{50}$ values at single-digit to sub-micromolar level.

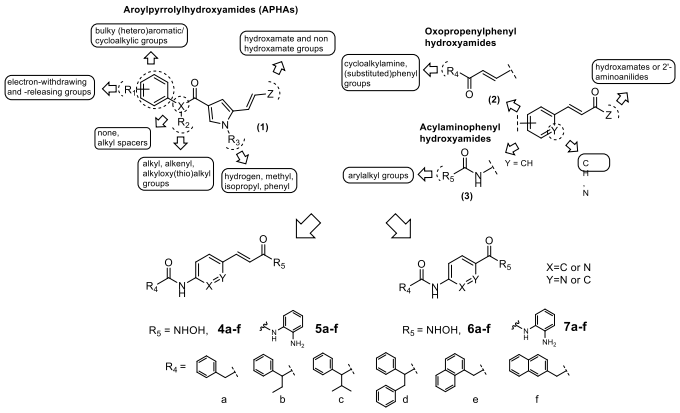


**Figure 1**

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PO-016

# FROM A HIT TO A PRECLINICAL DEVELOPMENT CANDIDATE: THE DISCOVERY OF ARN23765, A NOVEL PICOMOLAR CORRECTOR OF THE F508del-CFTR PROTEIN

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Cystic Fibrosis (CF) is a lethal genetic disease caused by mutations in the CF Transmembrane conductance Regulator (CFTR) chloride channel, resulting in reduced anion conductance on epithelial cells of multiple organs. Among the ca. 2000 mutations of the CFTR gene, the most frequent is the deletion of phenylalanine at position 508 (F508del).[1] This mutation causes a severe defect in protein folding and stability, and affects the gating behavior. An effective treatment for F508del CF patients requires at least a *corrector*, to increase CFTR levels at the cell surface, and a *potentiator,* to increase the opening frequency of the mutant CFTR channel.[2] Only few correctors for the treatment of CF patients bearing the F508del-CFTR mutation have been so far approved in combination with a potentiator. Trikafta®, a recently approved combination of two correctors, i.e., tezacaftor and elexacaftor, and the potentiator ivacaftor, represents an important step forward in the treatment of CF. However, there is still the need to continue developing new CF therapies.
Using a high-throughput functional phenotypic assay, based on the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP),[3] a set of about 15,000 maximally diverse commercial small-molecules was screened in two different cell types (FRT and CFBE41o-) stably expressing F508del-CFTR.

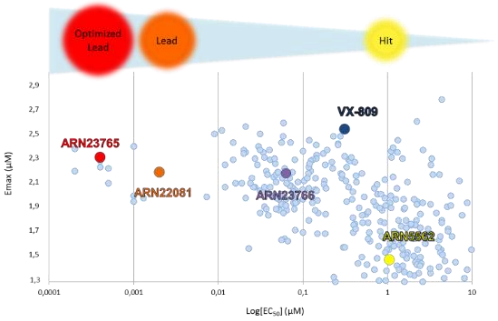


**Figure 1. SAR evolution to the discovery of ARN23765**

Starting from primary hit *ARN5562*, more than 500 analogues were synthesized and tested in the HS-YFP assay. Rounds of chemical modifications and functional evaluation in different secondary assays provided the information to build the Structure-Activity Relationships (SARs) within this novel chemical class. Based on its potency and efficacy in all cell-based assays, *ARN22081* was identified as the lead compound, and used as starting point for further SAR evolution. More advanced analogues were then identified having a suited drug-like profile for further development. This work allowed the discovery of ***ARN23765***[4] as a novel, potent CFTR corrector that is currently under preclinical development investigation.

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PO-017

# NLC AS PROMISING PLATFORM TO DELIVER NEW PRODRUGS IN THE TREATMENT OF OPHTALMIC CANCER

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Among ocular malignant tumors in adults, the most common is uveal melanoma (UM), characterized by a great ability to metastasize and by a 50% mortality in a year[1]. The role of both σ receptors in the development of the tumor suggests a possible involvement of $\sigma_1$ antagonist as a possible UM treatment approach; moreover, also HDAC demonstrated to exploit an important role in tumor proliferation, thus HDAC inhibitors could be potential adjuvants in therapy. Basing on these considerations, (±)-MRJF22, (*S*)- (–)-MRJF22 and (*R*)-(+)-MRJF22 – prodrugs of (±)-haloperidol metabolite II conjugated with valproic acid – demonstrated to have a promising antiangiogenic activity on 92-1 uveal melanoma (UM) cells[2]. Since ophthalmic administration continues to be a challenge for drug's bioavailability, the encapsulation into drug delivery systems, and in particular into nanostructured lipid carrier (NLC), represent a valid approach to overcome ocular drawbacks[3] and efficiently convey the drug to the target site. In this research work, NLC composed of Softisan and Isopropyl myristate and loaded with (*S*)-(–)-MRJF22 and (*R*)-(+)-MRJF22 were developed, obtaining respectively *S*-NLC and *R*-NLC. Phase Inversion Temperature method was selected as preparation method after the ascertainment of the thermal behavior of the two drugs using Differential Scanning Calorimetry (DSC). The characterization of the produced nanosystems involved the measurement of pH and osmolality values, which were into ocular physiological range due to the use of TRIS buffer as aqueous medium. Moreover, Photon Correlation Spectroscopy (PCS) analysis was performed in order to assess mean particle size and homogeneity (polydispersity index), which were respectively below 150 nm and 0.230; zeta potential resulted to be almost neutral (about -5 mV). Encapsulation efficiency (EE%) resulted to be 57.17% for (*S*)-(–)-MRJF22 into *S*-NLC and 55.87% for (*R*)-(+)-MRJF22 into *R*-NLC. Finally, *in vitro* cytocompatibility of blank and loaded NLC was analyzed on 92-1 UM cell line, demonstrating a good cell viability at concentration below 5 µM. The obtained results suggest that the platform developed for the delivery of (*S*)-(–)-MRJF22 and (*R*)-(+)-MRJF22 could be promising for the treatment of UM. Further studies aimed to the assessment of cellular uptake and *in vivo* eye biodistribution using fluorescent probe will be performed.

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PO-018

# EXTRACTION, PURIFICATION AND BIOACTIVITY OF POLICOSANOLS FROM NON-PSYCHOACTIVE *CANNABIS SATIVA* L.

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Policosanols (PCs) refer to a mixture of long-chain aliphatic alcohols with the number of carbon atoms varying from 22 to 34. These long-chain fatty alcohols are arousing great interest, given their multiple biological activities[1]. PCs are mostly known for their serum lipid and cholesterol-lowering effects through HMG-CoA reductase gene modulation, but also other properties are described in the literature for PCs, including antioxidant, anti-inflammatory and anti-proliferative activities [1,2]. There exist a large number of natural sources from which PCs can be extracted; of great relevance as PCs sources are sugar cane, rice bran, wheat and beeswax, their long-chain alcohol composition varying according to the specific raw material. Beside the aforementioned natural sources, recently fibre-type *Cannabis sativa* L. (hemp) has been identified as a potential novel and unexplored source of PCs, with a different and peculiar composition in fatty alcohols with respect to the other natural sources[3].

In the light of all the above, the aim of this project was the development of a highly efficient method for the extraction, purification and analysis of PCs in hemp, as well as the *in vitro* evaluation of their biological activities. In this research project, the wax material obtained from the supercritical-fluid extraction (SFE) with $CO_2$ from non-psychoactive *Cannabis sativa* L. inflorescences (hemp), was investigated, which is a byproduct obtained from cannabidiol purification process. To the best of our knowledge, this is the first study aimed at the characterization of PCs in hemp-wax. A microwave-assisted (MAE) procedure was used for transesterification and hydrolysis of the compounds of interest from the starting material, as PCs are usually present as long-chain esters in nature[2]. PCs were purified from reaction mixture by means of preparative liquid chromatography under normal phase conditions. In addition, a new HPLC method with evaporative light scattering detection (ELSD) was developed in order to efficiently quantify PCs in the final purified mixture. The purified PCs mixture was found to be rich in $C_{26}$OH and $C_{28}$OH. interestingly, odd-chain PCs (i.e. pentacosanol, $C_{25}$OH, and eptacosanol, C27OH) were also identified.

The new HPLC-ELSD method developed was applied also for the quantitative analysis of PCs in thirteen hemp inflorescences samples belonging to different varieties for scouting purposes.

*In vitro* assays were carried out on the purified PCs mixture obtained from hemp-wax, in order to assess their cholesterol-lowering, anti-inflammatory and antiproliferative properties

This study demonstrated the relevance of hemp-wax as a novel source of PCs and it will pave the way toward its future possible application in the pharmaceutical and nutraceutical ambits, in a circular economy perspective.

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*PO-019*

# TARGETING THE DIMER-MONOMER EQUILIBRIUM OF THYMIDYLATE SYNTHASE, TO ACCELERATE PROTEIN DEGRADATION AND CANCER CELL GROWTH INHIBITION

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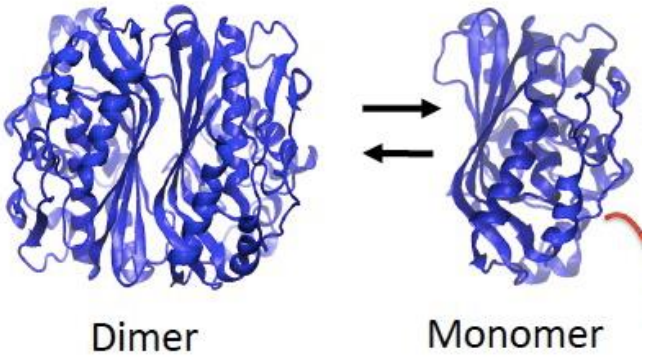
Thymidylate synthase (hTS) is an homodimeric protein existing in a dimer-monomer equilibrium. This equilibrium can be altered when specific ligands can bind at the protein interface. Drugs that target the hTS are widely used in anticancer therapy. However, treatment with classical substrate site-directed TS inhibitors such as 5fluorouracil and others, induces, among other mechanisms, protein over-expression and the development of drug resistance. Previous results suggest that the monomeric form of the protein is involved in process leading to protein level regulation. We expect that dimer to monomer shift can cause protein level reduction and anticancer efficacy. To discover interface binding hTS inhibitors that can shift the equilibrium to the monomer and reduce drug resistance development, we started a tethering-based approach targeting the interface residues. We mutated the tyrosine residue to cysteine and developed a disulfide covalent library screening. Then a medicinal chemistry program delivered an optimized lead. By combining structural, spectroscopic and kinetic investigation of the effects of the small molecules we confirmed the dissociative mechanism towards the hTS target. Then we showed that the best inhibitor, **E7,** accelerates the proteasomal degradation of hTS in cancer cells. **E7** showed a superior anticancer profile to 5fluorouracil in a mouse model of human pancreatic and ovarian cancer. Thus, over sixty years after the discovery of the first TS prodrug inhibitor, fluorouracil, **E7** breaks the link between TS inhibition and enhanced expression in response, providing a strategy to fight drug-resistant cancers. [1]

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PO-020

# FLUORESCENT NANODRUGS FOR NEUROINFLAMMATION INDUCED BY COVID-19

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Although Coronavirus Disease (COVID-19), a SARS-CoV-2 virus-derived infection, has long been described as a respiratory disease, numerous neurological complications have been highlighted, including the neuroinflammatory syndrome.[1] An obstacle to the neuroinflammation treatment is represented by the blood-brain barrier (BBB) since many anti-inflammatory drugs are unable to cross it. The use of nanoscale vectors could allow overcoming the limits of the administration of these drugs to the central nervous system (CNS).[2] In fact, nano-sized vectorization is a strategy currently used to improve the targeting and delivery of compounds for therapeutic use to CNS. It allows overcoming several of the limitations of conventional drug delivery systems such as non-specific biodistribution and targeting, reduced aqueous solubility, poor oral bioavailability and low therapeutic index.[3] Drugs resistance could also be solved, or at least reduced, by using nano-sized particles, which are able to accumulate in cells without being recognized by cell membrane efflux pumps, one of the main mediators of multi drug-resistance, with a consequent increase in the intracellular concentration of drugs.[4] To date, one of the main nano-sized particle formulation consists of covalent bonding of the nanoparticles with drugs, resulting in the formation of a prodrug. This strategy can also offer a delayed drug effect since covalent nano-prodrugs show greater stability than nanoparticles obtained through other strategies (e.g. drug encapsulation).

For this purpose, soft fluorescent organic nanoparticles (FONPs) have been prepared which, in addition to having a remarkable solubility in aqueous media, exhibit bright blue fluorescence properties (Figure 1A and 1B).[5] Furthermore, FONPs surface can be grafted also with hydrophobic drugs and fluorochromes (i.e., Nile Blue, Cyanine)[6] (Figure 1C). Such a conjugation would increase both the solubility and biodistribution of hydrophobic drugs and provides probes that, for their fluorescent properties, can be studied both *in vitro* and *in vivo* as an attempt to identify a possible treatment of COVID19- induced neuroinflammation.

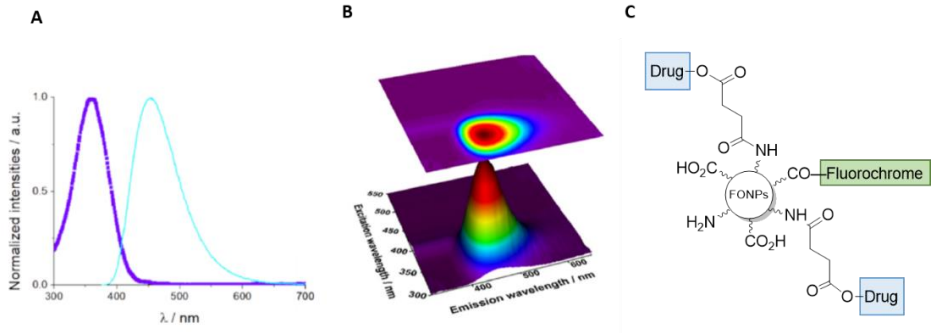


**Figure 1.** (A) Normalized absorption (violet) and emission (light blue) spectra of nanoparticles in water; (B) normalized 3D excitation and emission spectra of nanoparticles in water; (C) General chemical structure of FONPSs binding drugs and/or a fluorochrome.

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PO-021

# DEVELOPMENT OF NOVEL NONCOVALENT INHIBITORS OF 20S PROTEASOME AS ANTICANCER AGENTS

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The ubiquitin-proteasome system (UPS) is a key pathway involved in the intracellular protein turnover of eukaryotic cells. It is also responsible for the regular cell progression, immune surveillance and homeostasis control. Problems related to the UPS can lead to an uncontrolled cell proliferation and to tumor development. Proteasome is also involved in the degradation of many proto-oncoproteins, which, if not removed from cells, can generate malignancies.[1]

20S proteasome core is characterized by a barrel-like structure with four stacked rings: two inner β-rings, containing the catalytic subunits, and two outer α-rings, whose function is to maintain a gate through which proteins enter the barrel-like structure. The catalytic subunits are β1, β2, β5, which are responsible for the caspase-like, trypsin-like and chymotrypsin-like activities, respectively. It is currently well demonstrated that targeting the β5 catalytic subunit is a promising strategy to develop novel anticancer agents both for solid and hematologic tumors.[1]

Our research team has been involved in the last years into the development of new pseudopeptides as covalent and noncovalent proteasome inhibitors.[2-4] In this work, we designed and synthesized a new series of amides **1a-h** as noncovalent inhibitors of constitutive proteasome (Figure 1). The structure of novel amides bears a pyridone scaffold at the P3 site, as bioisostere of a leucine residue, whose function is also to lock the peptide into the bioactive conformation; while the amino group in position 5 of the pyridone scaffold has been introduced with the aim to increase the polarity of the molecules.

Because of the lack of specificity at the P2 site, we initially introduced a glycine residue, while the amide group, at the P1 site, was functionalized with aliphatic or aromatic substituents endowed with hydrophobic properties, as requested for the accommodation into S1 site.

All the synthesized molecules were tested against constitutive proteasome to evaluate their inhibitory properties by means of fluorimetric assays. The results of this investigation will be presented and discussed.

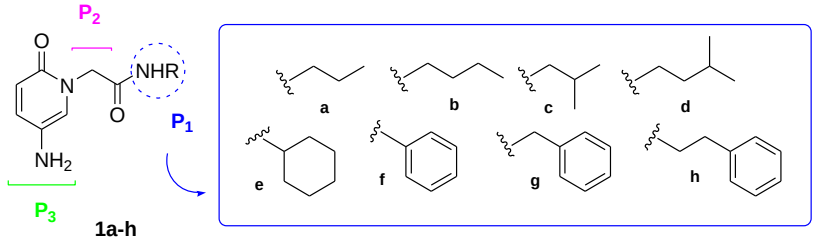


**Figure 1.** Structures of novel amides **1a-h**.

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PO-022

# TRIAZOLO-TRIAZINES AS PROTEIN KINASE CK1δ INHIBITORS: A FURTHER STEP TOWARDS THE DEVELOPMENT OF POTENTIAL NEUROPROTECTIVE AGENTS IN NEURODEGERATIVE DISORDERS

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Protein kinase CK1δ is known for its pleiotropic role in several physiopathological processes.[1] In particular, besides its well-known role in controlling the circadian rhythm, CK1δ is involved in the onset of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.[2] Thus, the lack of effective therapies has encouraged the development of new CK1δ inhibitors with a view to achieving innovative therapeutic approaches. The focus of this work was to investigate the triazolo-triazine-5,7-diamine scaffold to develop CK1δ ATP-competitive inhibitors since this nucleus has proven to be a successful replacement of the ATP's adenine ring able to interact with the catalytic domain (Figure 1). Particularly, taking advantage of computational studies on previous triazolo-triazine series[3], at the 7 position different diamine linkers were inserted to deepen the interactions with the solvent-exposed domain of the protein. Several alkyl and cycloalkyl diamine chains with different lengths were used but also the 1,4-bis(aminomethyl)cyclohexane to evaluate the role of the chain flexibility. All the substituents were easily inserted with microwave assisted synthesis. At the 5 position of the heteroaromatic system, a free amino group was maintained while at the 2 position the 2-thyenil group proved to be the most promising one. Interestingly, compounds bearing at the 7 position a diamine alkyl chain with the terminal amine protected by the *tert*-butyloxycarbonyl (BOC) group showed $IC_{50}$ values in the micromolar range. Notably, the most active compound (**1**, Figure 2) has the 1,4-bis(aminomethyl)cyclohexane as linker with an $IC_{50}$ value of 1.03 µM. These results allow us to assume a fundamental role of the reduced flexibility of the BOC-protected 1,4-bis(aminomethyl)cyclohexane chain and its spatial disposition in interacting with the kinase. In addition, considering the activity of the compounds with the terminal amine Boc-protected, the substitution of the terminal amino group with different aromatic systems or an acetyl group has been performed to study the role of the steric hindrance in this position as any additional interactions with the catalytic pocket. Unfortunately, the inserted groups have led to a worse or lacking inhibitory activity towards CK1δ. Building upon these findings, future computational studies will be able to elucidate the structure-activity relationship of this triazolo-triazine-5,7-diamines series leading to a more focused structural optimization.

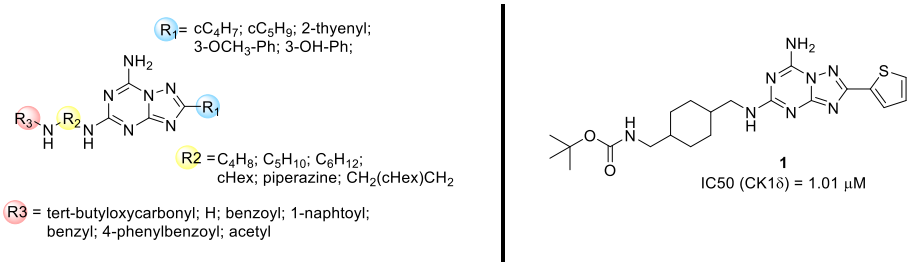


**Figure 1.** On the left: General structure of the triazolo-triazine-5,7-diamines synthesized as CK1δ ATP-competitive inhibitors. On the right: Chemical structure of the most potent compound of the series.

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PO-023

# NEW TRPM8 BLOCKERS EXERT ANTICANCER ACTIVITY OVER CASTRATION-RESISTANT PROSTATE CANCER MODELS

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TRPM8 has lately emerged as promising target in prostate cancer (PC), [1,2] so TRPM8 modulators has been suggested as potential anticancer agents in this malignancy. Their effectiveness has been recently demonstrated in a castration-resistant prostate cancer (CRPC) model, that is considered the most aggressive form of PC because it is usually resistant to androgen deprivation therapy (ADT). [3, 4] For this reason, the discovery of potent and selective TRPM8 modulators would improve the standardized treatments for PC currently being used. [5, 6] In this work, we describe the design and the synthesis of a new series of TRPM8 antagonists, firstly characterized in vitro for their potency and selectivity by fluorimetric calcium assays. This preliminary screening enabled to identify several selective and potent compounds (0.11 µM < $IC_{50}$ < 0.49 µM). Patch-clamp electrophysiological assays have been performed for the most potent derivatives, corroborating their notable activity. Furthermore, we have also investigated the behavior of these compounds in 2D and 3D model of PC. Again, the effectiveness of the compounds has been confirmed in spheroid models. All these results confirm a remarkable efficacy of these TRPM8 antagonists in inhibiting the growth induced by androgen in various PC cells and in CRPC models.

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PO-024

# DESIGN AND SYNTHESIS OF INNOVATIVE CHIMERA COMPOUNDS TARGETING TRANSTHYRETIN–Aβ POSITIVE CROSS INTERACTION

Ciccone, L.;[a] Tonali, N.;[b] Shi, C.; [b] Fruchart-Gaillard C[c].; Nencetti, S.[a]; Orlandini, E.[d,e], Kaffy, J.; [b] Ongeri, S. [b] and Rossello, A.[a,e]

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The aggregation of Amyloid Beta ($A\beta_{1-42}$) peptide acts as a critical early trigger in the etiopathogenesis of Alzheimer's disease (AD). An imbalance between production and clearance of Aβ in the brain results in spontaneous self-association into soluble toxic oligomers and insoluble aggregates.
Transthyretin (TTR) is another potential amyloid protein that establishes a positive cross-interaction with $A\beta_{1-42}$[1], avoiding its aggregation and participating in its clearance[2-4].
Human TTR is a homo-tetrameric protein characterized by four identical subunits o 14 kDa each. The four monomers, through hydrophobic interactions, are assembled in couples of dimers and two dimers are associated back to back to form a tetramer. The TTR tetramer is characterized by, two identical funnel-shaped named thyroxine binding sites (T4-BS), located at a dimer–dimer interface, figure 1. The tetramer is usually stable, exception when a single point mutation occurs and drastically decreases its stability, thus promoting amyloidosis. In contrast with its intrinsic amyloidogenic potential, as mentioned before, TTR can interact with Aβ and play a protective role in AD by sequestering Aβ and reducing protopathic stress. Alteration in TTR tetramer has been observed in AD patients. Studies suggest that the HF helix of TTR is crucial for the interaction with Aβ[5,6].
Rational multi-target drug design, which combine molecules having complementary modes of action, is a promising strategy for the development of new anti-AD drugs. In this contest, our aim is to combine through a linker a small molecule able to stabilizers TTR tetramer with a peptidomimetic, mimetic of TTR helix, to modulate the favorable cross-interaction between the two proteins and favouring Aβ scavenger[6] (figure 1).

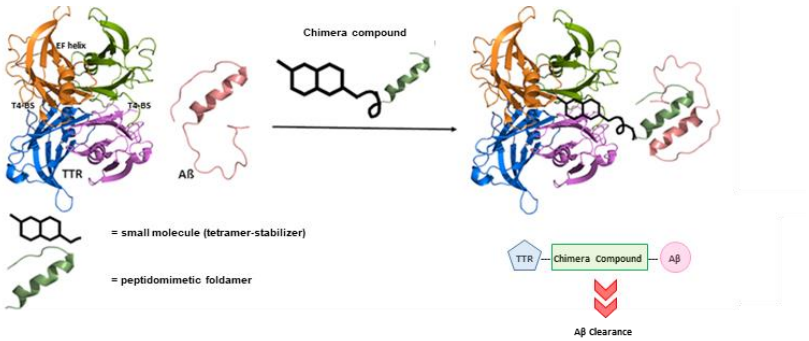


**Figure 1:** Graphical representation of chimera compounds

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PO-025

# COMBINED APPROACHES OF SUSTAINABLE CHEMISTRY AND TARGET FISHING FOR THE OPTIMIZATION OF PYRIDOBENZOTHIAZOLNES AS ANTI-FLAVIVIRUS AGENTS

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The mosquito-borne viruses belonging to the genus Flavivirus, such as Dengue (DENV), Zika (ZIKV), West Nile (WNV) and Chikungunya (CHIKV) viruses, are responsible of a high number of infections in humans, causing from mild flu-like symptoms to hemorrhagic fevers, hepatitis and neuropathies.[1] To date, no specific treatments are available for flavivirus diseases and new anti-flavivirus drugs are strongly needed.[1] Over the years, we identified a series of pyridobenzothiazolone (PBTZ) analogues as potent anti-flavivirus agents targeting the viral RNA polymerase.[2] Based on these results, in this work, we report the development of a sustainable three-component reaction (3CR) for the synthesis of functionalized PBTZ scaffolds at C-2 and C-8 positions. Then, by exploiting a one-pot procedure, we combined an in-situ hydrolysis with the 3CR affording PBTZ acid derivatives suitable for functionalization of C-4 position.[3] This new green procedure allowed us to quickly synthesize a wide set of PBTZ analogues as potential anti-flavivirus agents. Of note, we observed that the absence of a carboxylic function at the C-4 portion furnished analogues lacking polymerase inhibition activity, while showing a boosting in the cell-based anti-flavivirus activity, with compound **1** (Figure 1) exhibiting $EC_{50}$ values in the sub-micromolar range. Preliminary studies on the mechanism of action (MoA) suggested the ability of these analogues to generate fatal mutations in the new viral RNA. To perform more in depth investigations, we also synthesized a chemical probe, close analogue of **1**, to exploit a photo-affinity labeling approach for target identification. On the other hand, PBTZ analogues having a carboxylic function at C-4 position retained a significant DENV polymerase inhibition, but also gained a cell-based antiviral activity at the pre-entry step, as observed by time-of-addition experiments. Indeed, derivative **2** (Figure 1) exhibited a virucidal activity at low concentrations against a wide panel of RNA enveloped viruses (*i.e.* flaviviruses and SARS-CoV-2), highlighting a novel MoA for this series of derivatives.

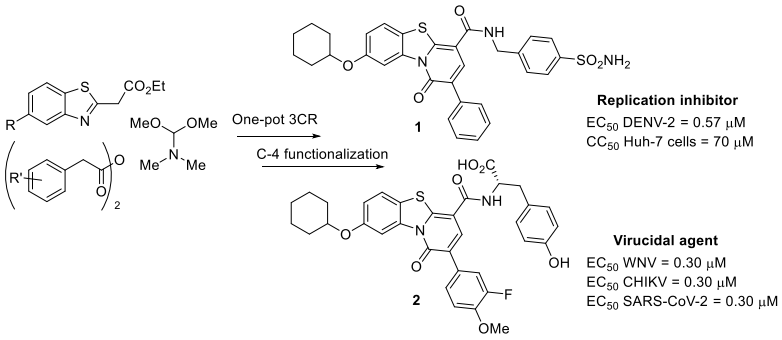


**Figure 1. One-pot 3CR and C-4 functionalization for the synthesis of anti-flavivirus and/or virucidal agents.**

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*PO-026*

# MULTI-OBJECTIVE OPTIMIZATION OF NEURAL NETWORKS FOR THE DE NOVO GENERATION OF TARGETED CHEMICAL LIBRARIES

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We present an adaptation of a SMILES artificial intelligence generative algorithm to the de novo generation of chemical libraries fulfilling multiple objective optimality.[1] The algorithm, originally by Olivecrona & al.[2], is based on neural networks. The adaptation plugs in the training iterative cycles of the network, where a scoring function addressing different objectives from a Paretian perspective is introduced: such objectives may range in a large number of physicochemical requirements, such as bounds on MW, logP, HBA, HBD and even synthesisability and affinity to chosen targets; this latter bias currently being introduced only be means of similarity constraints to well known ligands. The method has been tested applying it to the generation of chemical libraries targeting neuramidase, acetylcholinesterase and the main protease of severe acute respiratory syndrome coronavirus 2. The libraries generated by the proposed method have been extensively checked for validity, diversity, drug-likeness and respondence to the objectives. Finally, molecular docking has been used to check the capability of the method to effectively address the purpose of generating molecules with high affinity to selected targets. The implementation has been made available to the public as open source project at https://Github.Com/Alberdom88/Moo-Denovo.

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PO-027

# AN ANTIVIRAL STRATEGY AGAINST SARS-CoV-2 SPIKE PROTEIN BASED ON REDUCING AGENTS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic and currently, specific treatments for SARS-CoV-2 are still needed. The spike glycoprotein is located on the surface of the SARS-CoV-2 envelope and mediates virus entry by interacting with the host ACE2 receptor [1]. Recently, a large body of literature data has highlighted the critical role of disulfide bonds in the dynamic structure of the Spike protein in receptor binding and entry/fusion protein [2-4]. Preliminary experimental studies performed by some of us, have demonstrated how UV-C exposure of SARS-CoV-2 inactivates its infection capability. Mass spectrometry demonstrated that this occurs by the reduction of key disulfide bonds in the spike structure, while surface plasmon resonance (SPR) analysis confirmed that UV-C exposure causes a significant decrease in the spike binding affinity for ACE2.
Based on these premises, a model of SARS-CoV-2 spike protein [5] has been used to perform an accurate analysis of its dynamic behavior by an extensive molecular dynamics (MD) simulation at 500ns. Solvent Accessible Surface Area (SASA) calculations on the MD trajectory revealed that, among the targetable regions suggested by literature and by mass spectrometry, an intriguing transient pocket emerges as of interest that includes one of the disulfide bridges. A repositioning strategy has been consequently applied, screening with docking procedures an in-house database of drugs and traditional medicine compounds. 40 thiol-based small molecules have been filtered and, among them, 8 compounds have been selected as optimal candidates able to perform a disulfide bond with the cysteine residues of the target pocket. The stability of two of the eight molecules has been already evaluated by three MD simulations at 100ns, while calculations on the remaining six complexes are currently running. The most promising compounds identified will be presented and fully discussed. Computational simulations were supported by the EGI-ACE Horizon 2020 project that granted access to EOSC infrastructure and platform services and ELIXIR CNR.BiOmics, the research infrastructure for life-science data.
As a prosecution of the research, mass spectrometry and SPR evaluations will be performed on the selected compounds to ascertain their effective capacity to disrupt key disulfide bonds in the spike protein and their ability at inactivating SARS-CoV-2 infection will be tested *in vitro*.

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*PO-028*

# NUTRACEUTICAL BIODEVICES FROM EXTRA-VIRGIN OLIVE OIL BY-PRODUCTS AS INNOVATIVE AND SUSTAINABLE APPROACH OF WASTE RECYCLING IN FOOD SUPPLY CHAIN

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Nowadays, the nutritional and health-promoting properties of extra virgin olive oil (EVOO) is well recognized. A wide spectrum of nutraceutical properties has been attributed to EVOO, due to the presence of polyphenols, such as oleocanthal and oleacein.[1]
EVOO production shows great relevance from an environmental sustainability viewpoint due to difficulty of treatment of related waste products.[2] In fact, EVOO waste management is characterized by significant amounts of by-products such as a semisolid sediment residue, olive mill wastewater (OMW) and olive leaves obtained during the harvesting or pruning process of olive fruits. On the other hand, these discards contain phenolic compounds endowed with nutraceutical properties, a promising font of bioactive compounds to be valorized.[3]
Recently, the development of biomedical devices constituted by biocompatible fibers incorporating olive leaf extract (OLE) obtained by discarded leaves of olive trees have been reported (**Figure 1**).[4] These biodevices showed anti-inflammatory and antibacterial properties which are essential in wound healing and tissue regeneration.
The aim of this work is the further valorization of EVOO and its by-products (leaves, sediment and OMW), developing new valuable BioDevices endowed with nutraceutical properties in biomedical field. The project well aligns with the sustainable goals of Recovery and Resilience Plan (PNRR) concerning the re-evaluation of food waste supply chain, which significantly affects the environmental impact.

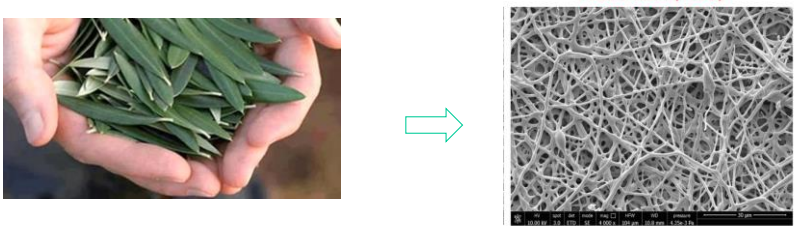

***Figure 1.*** *BioDevice based on olive leaf extract incorporated in biofibers*

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PO-029

# COMPUTATIONAL AND EXPERIMENTAL STUDIES OF WHEY PROTEIN-DERIVED SMALL PEPTIDES WITH TARGET-SPECIFIC ACTIVITY AGAINST THE SARS-CoV-2 MAIN PROTEASE

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The fight against the COVID-19 pandemic has represented on the greatest challenges facing the scientific community in the recent years. In these respect, three whey protein-derived peptides (IAEK, IPAVF, MHI), endowed with ACE inhibitory activity[1], were examined for their antiviral activity against the SARS-CoV-2 3C-like protease ($3CL^{pro}$) by employing reliable and detailed *in silico* analyses[2]. Computational studies showed consistent binding poses within $3CL^{pro}$ for the three investigated small peptides, considering docking scores as well as the binding free energy values. Validation by in vitro experiments confirmed these results. In details, IPAVF exhibited the highest inhibitory activity by returning an IC50 equal to 1.21 μM; it was followed by IAEK, which registered an IC50 of 154.40 μM, whereas MHI was less active with an IC50 equal to 2700.62 μM. Interestingly, none of the three peptides returned a reliable binding pose within Human Rhinovirus 3C protease ($3C^{pro}$) binding site, and neither registered inhibitory activity against $3C^{pro}$. The results herein open the door to new opportunities for the development of dual-target small peptides that are endowed with selective antiviral $3CL^{pro}$ and inhibitory ACE activities.

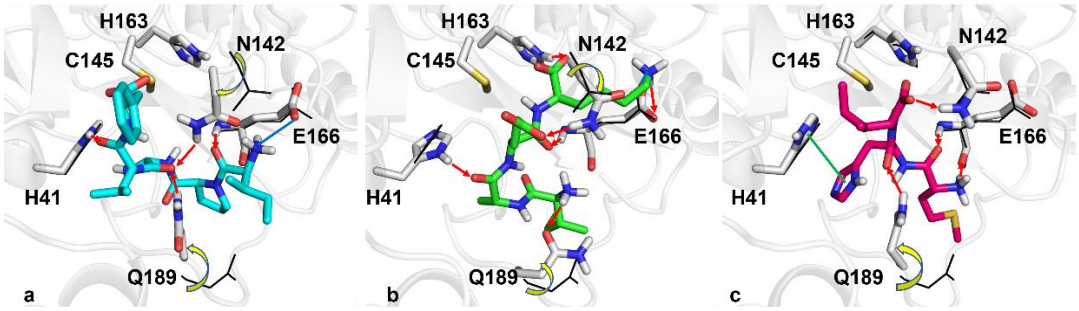


**Figure 1**. Panels (a–c) report the best pose returned from docking simulations for IPAVF (cyan sticks),IAEK (green sticks), and MHI (magenta sticks) peptides, respectively. Red arrows and green and blue lines depict hydrogen bonds, π–π, and electrostatic interactions, respectively. Black wireframes show the original side-chain conformation of the 7L0D crystal structures. Yellow arrows highlight the shifting of the side chains from their original positions, due to the induced fit.

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*PO-030*

# SYNTHESIS AND CHARACTERIZATION OF NEW $A_{2A}$ ADENOSINE RECEPTOR ANTAGONISTS AS POTENTIAL NEUROPROTECTIVE AGENTS

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Adenosine receptors, classified in $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ subtypes, are G-protein coupled receptors implicated in many functions both in central nervous system and in the whole body. The $A_{2A}$ subtype is of particular interest because it is involved in neuroprotective processes. It has been demonstrated that inhibition of $A_{2A}$ adenosine receptors exerts neuroprotective effects counteracting neuroinflammatory processes and astroglial and microglial activation. Many $A_{2A}$ adenosine receptor ligands were developed and some of them, with antagonist activity, resulted being effective in animal models of Parkinson's disease. Istradefylline, an $A_{2A}$ antagonist endowed with xanthine structure, have been approved in Japan and USA for the treatment of this disease used together with levodopa. The reference compound for the study of this receptor subtype is ZM 241385, an $A_{2A}$ adenosine receptor antagonist endowed with a triazolotriazine scaffold. In this work, based on the structures of ZM 241385 and of 8-substituted 9-ethyladenines, like 9-ethyl-8-furyladenine (**1**), a new series of 2,8 disubstituted 9-ethyladenine derivatives has been designed and synthesized (Figure 1).
The synthesis was performed using common organic chemistry reactions starting from commercial 2,6-dichloropurine. All the new compounds were tested on human recombinant adenosine receptors stably transfected on Chinese hamster ovary (CHO) cells using specific radioligands. Compound bearing the amino side chain present in the ZM 241385 and its p-methoxy analogue showed high affinities at $A_{2A}$ adenosine receptors, comparable to that of ZM 241385, but lower selectivity for $A_1$ and $A_3$ subtypes. Furthermore, one of them was found to exert anti-inflammatory properties in a microglial model of neuroinflammation, since it was able to reduce pro-inflammatory mediators and to increase anti-inflammatory cytokines in microglia cells.

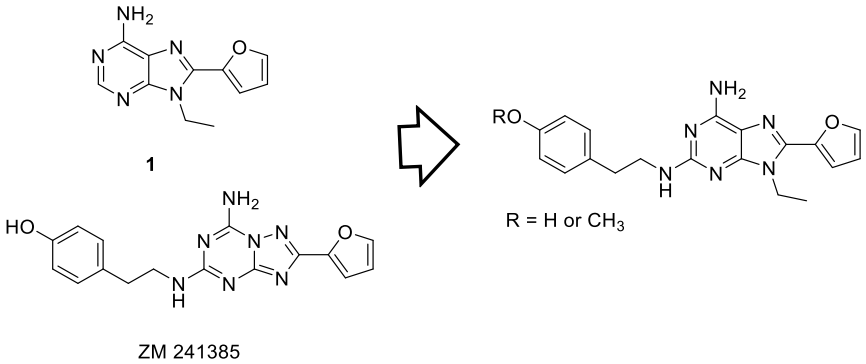


**Figure 1** Structure of 1, ZM 241385 and new synthesized molecules

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Spinaci, A.; Lambertucci, C.; Buccioni, M.; Dal Ben, D.; Graiff, C.; Barbalace, M.C.; Hrelia, S.; Angeloni, C.; Tayebati, S.K.; Ubaldi, M.; Masi, A.; Klotz, K.-N.; Volpini, R.; Marucci, G. *Molecules* **2022**, *27(8)*, 2386.

PO-031

# STRUCTURE-ACTIVITY RELATIONSHIP STUDIES LEADING TO SELECTIVE DOPAMINE D4 RECEPTOR LIGANDS POTENTIALLY USEFUL FOR THE TREATMENT OF GLIOBLASTOMA

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Glioblastomas (GBM) are the most common malignant brain tumors and are characterized by an aggressive invasive behavior associated with a survival median of 12-15 months and high resistance to therapy. Temozolomide is the only chemotherapeutic showing positive outcomes, but its efficacy is transient and occurs in a subgroup of patients.[1] Thus, the clinical need is to develop new specific anticancer agents targeting regulatory pathways outside the traditional chemotherapies. Dopamine and its receptors are involved in the growth of cancers and, in particular, D4 receptor subtype (D4R) has recently garnered interest as a potentially therapeutic target for GBM treatment, considering that inhibition of D4R disrupts the autophagy-lysosomal pathway of GBM neural stem cells, leading to apoptosis.[2] According to the Cancer Genome Atlas data on GBM gene expression, adult patients with high D4R levels have worse survival than those with low expression. However, at present, few data suggesting that the D4R is crucial for the survival of GBM cells are reported.
Based on these observations, in this study, new ligands endowed with high affinity and selectivity for D4R were discovered starting from the brain penetrant and D4R selective lead compound 1-(3-(4-phenylpiperazin-1-yl)propyl)-3,4-dihydroquinolin-2(1H)-one (**1**).[3] In particular, maintaining the *N*-arylpiperazine moiety, the quinolinone portion was replaced by bioisosteric nuclei and the propyl linker by chains of different lengths. Moreover, substituents with different electronic and lipophilic contributions were inserted in ortho-, meta- and para-position of the *N*-aryl terminal (Figure 1).

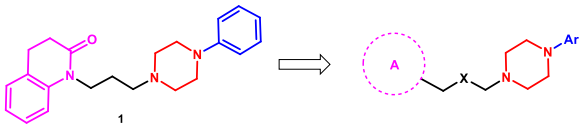


**Figure 1.** Modifications of the chemical structure of the lead compound **1**.

All the compounds were evaluated for their affinity at D2R, D3R and D4R by radioligand binding assays. The most selective D4R ligands were also tested for their functional activities by BRET assays to detect D4R G-protein activation and β-arrestin recruitment. Finally, the most interesting compounds were evaluated for their potential in affecting the viability of GBM cells lines and primary GBM stem cells. The potent and highly selective D4R ligands emerged from this study might further shed light on the role played by this subtype in GBM and, especially, become lead compounds for the discovery of new alternatives to the standard treatments such as surgery and radiotherapy, that cannot always be applied, and pharmacological treatments, that are still very limited because of drug resistance.

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*PO-032*

# IPEROXO/DEQUALINIUM AND W84/DEQUALINIUM HYBRID LIGANDS: SYNTHESIS, PHARMACOLOGICAL AND COMPUTATIONAL INVESTIGATION AT $M_2$ mAChRs

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The use of bitopic ligands to study the properties of Muscarinic Acetylcholine Receptors (mAChRs) is a very active field of research.[1,2,3] In this regard, bipharmacophoric molecular probes were found to switch between two different binding orientations in the $M_2$ subtype, resulting in both active and inactive populations of receptors bound by a given ligand, a behavior that has been termed dynamic ligand binding.[2] Continuing our interest in this field, we focused our attention on the properties of Dequalinium chloride, a bis-pyridinium quaternary ammonium compound, which was recently reported to act as a potent muscarinic allosteric modulator, showing an overall selectivity towards the $M_2$ subtype.[4] Starting from this observation, we designed and synthesized two series of novel hybrid ligands incorporating in their molecular skeleton the allosteric moiety of Dequalinium (**Figure 1**). The first series is based on the combination of Dequalinium with the orthosteric superagonist Iperoxo and aimed at novel chemical probes interacting cooperatively with both the orthosteric and the allosteric binding site. In the second series, Dequalinium was hybridized with the allosteric modulator W-84, affording new putative allo/allosteric ligands.

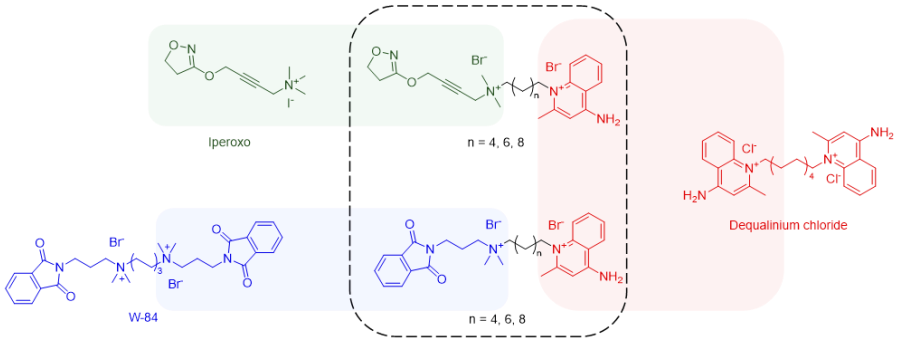


**Figure 1:** The new Dequalinium hybrid ligands.

In this study, the newly synthetized compounds have been characterized through competitive binding assays at the five mAChR subtypes. The activity on the allosteric site has been investigated by measuring the affinity ($logK_{occ}$) at the [$^3$H]-NMS-occupied muscarinic $hM_2$ subtype. Moreover, docking simulations have been performed to highlight the interactions within the ortho/allosteric binding pockets of the $hM_2$ crystal structure addressing the role of linker length. The synthetic approach and the details of the pharmacological and computational investigations will be illustrated and discussed.

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PO-033

# INSIGHTS ON HETEROCYCLIC SULFONAMIDES AND SULFAMIDES AS CARBONIC ANHYDRASE INHIBITORS FOR TREATING CANCER DISEASES

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Carbonic anhydrases (CAs) are $Zn^{2+}$-containing enzymes catalyzing the reversible hydration of carbon dioxide to generate bicarbonate and proton. There are distinct CA isoforms that are implicated in a wide range of diseases, including cancer. Particularly, in solid tumors the isoforms hCA IX and hCA XII regulate pH thus controlling the differential pH microenvironment within cancer cells; as a result, the microenvironment is favorable for cell survival under stressful conditions. Based on this evidence, selective inhibition of hCA IX and/or hCA XII isoforms could offer amenable molecular targets for development of theranostic in cancer therapy.[1] In recent years, many new advances have been made toward the development of CA inhibitors bearing sulfonamide or sulfamide moiety binding the zinc ion present in the active site. In addition to the above-mentioned crucial chemical features, these CA inhibitors are characterized by structural fragments able to create a network of interactions in the CA cavity as well as to address selectivity toward hCA IX/XII isoforms over ubiquitous hCA I/II. Searching for new active and selective inhibitors, we have rationally designed a small series of compounds that demonstrated high affinity towards tumor expressed hCA IX/XII isoforms.[2-4] Encouraged by these promising results, in this study we extended our exploration toward a new series of sulfonamides/sulfamides (Figure 1) that were synthesized and tested in a stopped-flow $CO_2$ hydrase assay. To better understand the mechanism of inhibition and isoform selectivity we performed theoretical and structural studies, that described the recognition process within catalytic binding site (Figure 1).

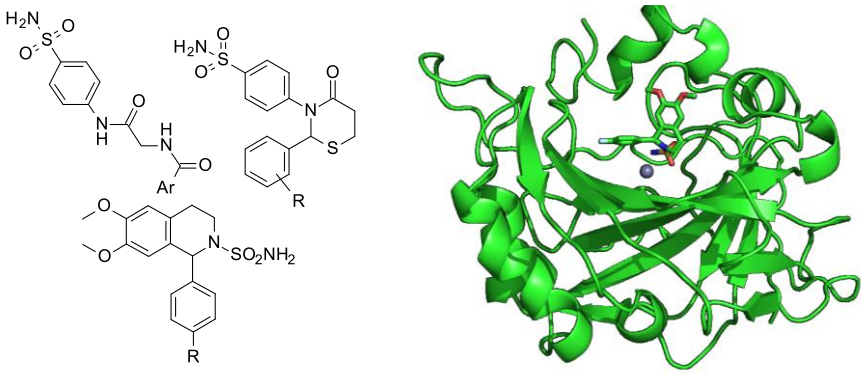


**Figure 1**. Chemical structures of sulfamide/sulfonamide-based compounds and recognition within CA cavity

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PO-034

# SMALL MOLECULE INHIBITOR OF ALPHA-SYN AGGREGATION: PREDICTION OF PUTATIVE BINDING SITE AND MOLECULAR DOCKING

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Alpha-synuclein (α-syn) is a highly disordered neuronal presynaptic protein, that physiologically regulates the release of neurotransmitters. However, α-syn accumulation is the main histopathological feature of some neurodegenerative disorders, called synucleinopathies. Among them, in Parkinson's disease (PD), the α-syn accumulates mainly in Lewy's bodies and Lewy's neurites.[1] Therefore, the identification of small molecules able to inhibit α -syn aggregation could be a viable treatment for PD.

To achieve this goal, the webserver SwissSimilarity was used to perform a ligand-based virtual screening employing two promising α-syn aggregation inhibitors as query compounds[2,3]. Three different approaches have been independently employed to estimate the similarity between the reference molecules and those contained in the SPECS library, used as search database: FP2 fingerprints, Spectrophores and Electroshape. Thirty-five hits were selected for the *in vitro* studies based on their score, drug-likeness properties and structural diversity, allowing us to identify MESC-4 derivative as potent α -syn aggregation inhibitor in Thioflavin T fluorescence assay. To investigate how MESC-4 binds to α-syn, we performed a computational strategy to map putative druggable pockets using a combination of three different software, such as fPocket, SiteMap and FTMap. To perform the planned computational analysis, we studied two structures of the α -syn fibrils available on RCSB PDB databases (PDB code: 6FLT and 2N0A); through a clustering approach we obtained two possible druggable sites, called SiteA and SiteB. (Fig.1 A). Using three different software (Autodock, Gold, Glide), a consensus docking methodology was performed to explore the binding ability of MESC-4. As result, we can suggest that MESC-4 preferably binds the SiteA rather than to SiteB. (Fig.1B).

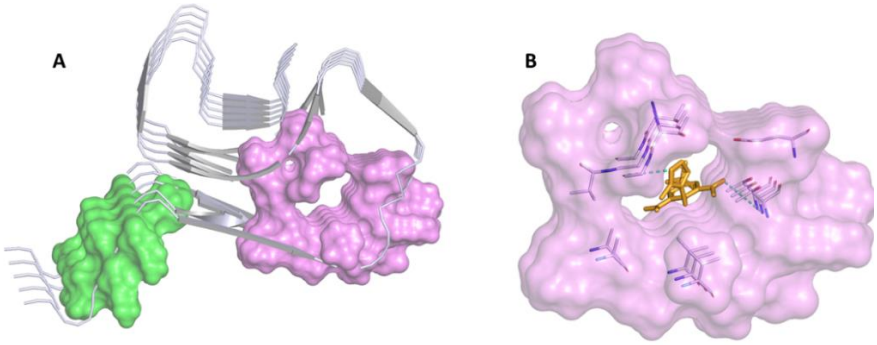


**Figure 1: (A)** Putative binding pockets highlighted in different color surfaces, in magenta SiteA and in green SiteB. **(B)** Plausible binding mode of MESC-4 (orange sticks) into the binding site A.

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PO-035

# DUALSTERIC MOLECULES TARGETING AROMATASE ENZYME FOR BREAST CANCER TREATMENT

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One of the first-line therapies for the treatment of hormone-dependent breast cancer is based on suppression of estrogen production, through the inhibition of cytochrome P450 aromatase (AR), the key enzyme for estrogen biosynthesis. Despite the remarkable efficacy of marketed aromatase inhibitors (AIs), serious side-effects and drug resistance can arise, limiting their therapeutic application. The need to overcome the different issues related to the use of these drugs has led to investigate other potential mechanisms of inhibition of estrogens production. Recently, allosteric inhibition of AR has emerged as an intriguing alternative strategy, since three putative binding sites in different regions of AR structure were identified.[1] Among these, one was located close to the most favorable access channel for the catalytic site. As part of a project aimed at the development of novel AIs, we recently made a first attempt to develop dual-mode inhibitors,[2] in which a rigid pentinyloxy chain, potentially able to reach the access channel, was inserted on some imidazolylmethylxanthones[3] that had previously shown orthosteric inhibition of AR. Unfortunately, the presence of this long and rigid side chain on the xanthone core did not lead to higher activity. Thus, to get further information about the chemical space of the putative allosteric site and establish the most favorable structural features to interact with the residues lining the enzyme access channel, new dualsteric compounds have now been designed with improved central core flexibility (Figure 1). To this aim, the rigid xanthone core was replaced by a 3- or 4-imidazolylmethylbenzophenone scaffold, already reported by us in the structure of compounds endowed with AR inhibiting activity, and alkoxy side chains of different length and rigidity were inserted in position 4'. Among the two series of compounds, 3-imidazolylmethylbenzophenones proved to be remarkably more potent than the corresponding 4-substituted derivatives, and the side chain also proved to play a role in activity. Classical molecular dynamics (MD) and mixed quantum-classical (QM/MM) MD simulations were performed to rationalize the binding mode of this set of compounds, along with the key interactions with enzyme residues.

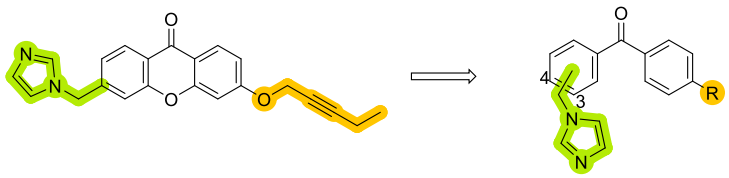


**Figure 1.** Design of novel benzophenones.

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PO-036

# QSPR STUDY: MULTIVARIATE APPROACHES IN THE ASSESSMENT OF THE PHOTOSTABILITY OF NEWLY SYNTHESIZED DIHYDROPYRIDINES

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1,4-dihydropyridines (DHPs) represent the most important class of L-type calcium channel blockers, widely used in the treatment of cardiovascular diseases, particularly hypertension and angina pectoris.[1] Recently, modifications of the DHP scaffold have modulated the channel blocking activity towards the T-type calcium channel allowing to obtain active molecules in treating a further variety of disorders, including pain and epilepsy.[2] DHPs are characterized by a high tendency to degrade when exposed to light, leading to an oxidation product deriving from aromatization of the dihydropyridine ring.[3] In this study, the elaboration of a quantitative structure-property relationships (QSPR) model has been carried out by correlating the light sensitivity of sixteen newly synthesized DHPs against theoretical molecular descriptors. Photodegradation experiments were performed by exposing the drugs to a Xenon lamp following the ICH rules. The degradation was monitored by spectrophotometry and spectral data were elaborated by Multivariate Curve Resolution (MCR) methodologies.[4] The data modelling suggests that most of the tested compounds furnished a pyridine derivative as the only photodegradation product, according to a first-order kinetic. On the other hand, for some compounds, together with the pyridine derivative, the formation of a secondary photoproduct has been observed. The kinetic rates calculated for photodegradation experiments were combined with a series of descriptors related to the chemical structures calculated by a dedicate software.[5] After an accurate variable selection, 15 molecular descriptors were used in the elaboration of the QSPR model (Fig.1) to correlate the kinetic photodegradation rate to the molecular structure.

This model was validated with an external set of novel DHPs, yielding very satisfactory statistical results. The good agreement between the predicted and measured photodegradation rate demonstrated the high accuracy of the QSPR model in predicting the photosensitivity of the drugs belonging to this class. Accordingly, the calculated model could represent an effective tool to design new analogs characterized by higher photostability.

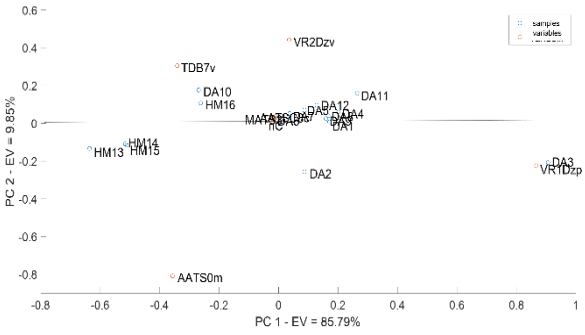


**Figure 1: Scores and loadings bi-plot of the QSPR model**

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PO-037

# LETROZOLE STABILITY STUDY BY MULTIVARIATE ANALYSIS AND STRUCTURE-BASED MOLECULAR DOCKING

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Letrozole (LTZ) is one of the most prescribed drugs for the treatment of breast cancer in post-menopausal women, and it is endowed with selective peripheral aromatase inhibitory activity. The efficacy of this drug is also a consequence of its long-lasting activity, likely due to its metabolic stability. The reactivity of cyano groups in the letrozole structure could, however, lead to chemical derivatives still endowed with residual biological activity. Herein, the chemical degradation process of the drug is studied by coupling multivariate curve resolution and spectrophotometric methodologies in order to assess a detailed kinetic profile. Degradation studies indicate that LTZ, although stable to light irradiation, generates three main derivatives under stressing chemical conditions, such as changes in pH or oxidizing environment. The analytical data collected by UV/Vis spectrophotometry during the kinetic experiments are processed by chemometric methodologies (Fig. 1, left).[1] The soft multivariate curve resolution of the data matrices confirms the role of pH and oxidants in favoring the formation of the diamide, diacid and N2-oxide degradation products. The capability of these compounds to accommodate into the active site of the enzyme has been investigated by molecular docking (Fig. 1, right).[2] Our results suggest that the sustained inhibitory activity of letrozole may be at least in part attributed to the degradation compounds since the metabolism of LTZ could lead to compounds still effective.

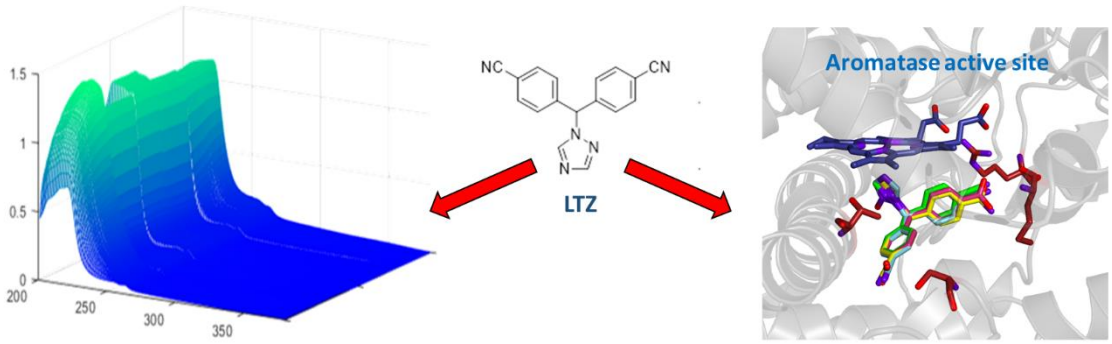


**Figure 1. LTZ degradation experiments monitored by UV/Vis analysis and molecular docking of degradation products.**

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PO-038

# DESIGN, SYNTHESIS MOLECULAR MODELING AND PHARMACOLOGICAL EVALUATION OF 2,7-DIAZASPIRO[4.4]NONANE DERIVATIVES AS NOVEL SIGMA RECEPTORS LIGANDS

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Sigma receptors (SRs) represent a unique receptor class involved in several biological and pathological conditions. Two subtypes are distinguished and termed sigma-1 receptor (S1R) and sigma-2 receptor (S2R), having different structure, biological functions, and pharmacological profile. The S1R is highly expressed in both central and peripheral nervous system, in areas of great relevance for neuroprotection, neuroinflammation, neurotransmission, and neuroplasticity. Alterations in the function of S1R have been associated with neurodegenerative diseases, pain, stroke, and retinal degeneration.[1]
Over the years, spirocyclic compounds have gained increasing interest in the development of bioactive compounds and contribute to a variety of approved drugs and drug candidates.[2] The introduction of a spirocyclic moiety in a molecule grant a peculiar spatial arrangement that may influence important parameters, such as potency, selectivity and physicochemical properties.
Here we report the development of novel 2,7-diazaspiro[4.4]nonane derivatives where the central core has been flanked with hydrophobic groups at a certain distance to the central basic amine being this a common structural requirement of potent SR ligands as identified by previous works (Figure 1).

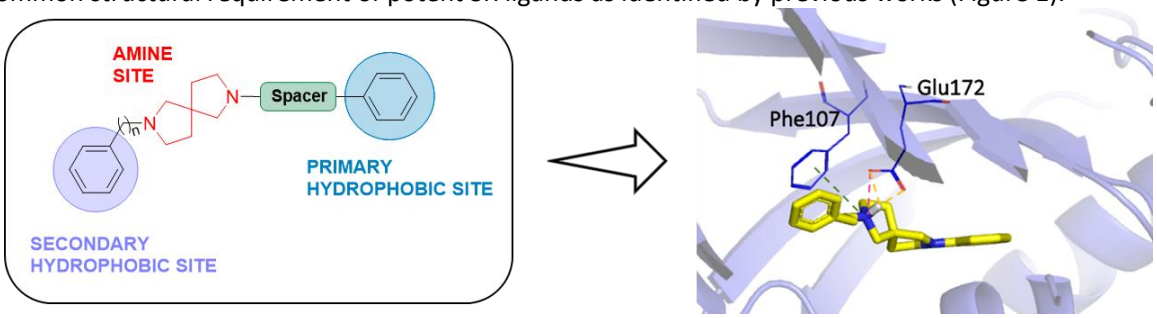


**Figure 1.** General structure of 2,7-diazaspiro[4.4]nonane SR ligands.

This chemical matter has been developed according to structure-affinity relationships approach consisting in the following steps: (i) design of new candidate ligands; (ii) in vitro radioligand binding assays; (iii) iterative compounds design based on affinity and selectivity; (iv) synthesis of the new compounds for further pharmacological evaluation. Several compounds have been synthetized and detailed mechanistic studies performed to understand the binding with the SR. Molecular modeling analysis was carried out to deeply analyze the binding mode and the interactions established between the ligands and S1R and S2R. Finally, the most notable compounds have been subjected to toxicity evaluation and thus screened for phenotypic effects in in vitro and in vivo models.

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PO-039

# *N*-ADAMANTYL-ANTHRANIL AMIDE DERIVATIVES: NEW SELECTIVE LIGANDS FOR THE CB2 RECEPTOR, A POSSIBLE THERAPEUTIC TARGET AGAINST COVID-19

Graziano, G.;[a] Delre, P.;[b] Jose, M. B.;[c] Carofiglio, F.;[a] Gioè, C.;[d] Majellaro, M.;[d] Nicolotti, O.;[a] Colabufo, N. A.;[a] Abate, C.;[a] Loza, M. I.;[c] Sotelo, E.;[d] Ligresti, A.;[e] Kostrzewa, M.;[e] Riganti, C.;[f] Mangiatordi, G. M.;[b] Contino, M.;[a*] Stefanachi, A.;[a*] and Leonetti, F.[a]

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Cannabinoid receptor 2 (CB2R) is a GPCR that, together with cannabinoid receptor 1 (CB1R), endogenous cannabinoids and enzymes for their metabolism, forms the EndoCannabinoid System (ECS). Several studies have shown that CB2R is involved in pathological states, such as neurodegenerative disorder, neuropathic pain, cells and in inflammation associated with obesity, insulin resistance, and non-alcoholic fatty liver disease. For this reason, the anti-inflammatory and immune-modulatory actions of CB2R ligands are emerging as a novel therapeutic option for these diseases.
In late December 2019, a novel coronavirus (SARS-CoV-2 or CoV-19) appeared in Wuhan, China, causing a global pandemic. SARS-CoV-2 causes mild to severe respiratory tract inflammation. The stimulation of CB2 receptors is known to limit the release of pro-inflammatory cytokines, shift the macrophage phenotype towards the anti-inflammatory M2 type and enhance the immune-modulating properties of mesenchymal stromal cells. For these reasons, CB2R may also be a therapeutic target against COVID-19.[1]
Unfortunately, CB2R has a high degree of homology with CB1R. We aimed to identify new selective CB2R ligands as potential drugs devoid of psychotropic side effects caused by CB1R activation. Supported by computational studies performed on the recently released CB2R crystal structures in complex with a synthetic antagonist, AM10257,[2] we focused our attention on the design and synthesis of new N-adamantyl-anthranil amides (General structure illustrated in figure 1B), ligands selective for CB2R.

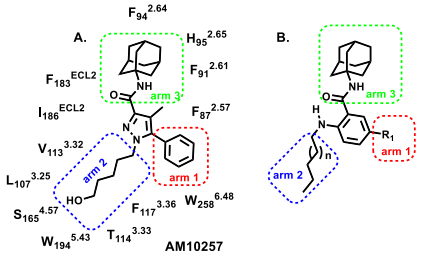


**Figure 1,** Comparison between "three-arms pose interactions" of antagonist (AM10257) on CB2R binding pocket [2] (A) and the general structure of our *N*-adamantyl-anthranil amide derivatives (B).

We explored various substitutions on the indicated three arms as to identify a rational SAFIR and SAR model. The interesting results from in vitro functional assay and from the cytokines production highlighted the pivotal role of arm1 substitution on the CB2R activity profile (switch from agonist to antagonistic activity).

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PO-040

# DEVELOPMENT OF A MICROSCALE THERMOPHORESIS-BASED METHOD FOR SCREENING AND CHARACTERIZING INHIBITORS OF THE METHYL LYSINE READER PROTEIN MRG15

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MRG15 is a transcription factor with a crucial role in embryonic development, cell proliferation and cellular senescence.[1,2] Structurally, MRG15 contains, among all, a methylation reader domain (chromodomain) which responsible for the interaction of the protein with histone and non-histone substrates. Despite MRG15 is involved in different physiological and pathological states, to date the role of this protein has not been fully elucidated due to the lack of specific and potent chemical probes. Therefore, as for other reader proteins, the interest in developing small-molecule inhibitors is very high.[3]

Here we report the development of a Microscale Thermophoresis (MST) assay for the reader protein MRG15 (Figure 1). In the first part of the work, the labeling procedure of the protein was optimized, and an assay development was applied, testing different buffers and the effect of stabilizing agents on MST signal. Subsequently, assay validation was performed using the reference compound UNC1215. Finally, the screening of a small library of compounds was performed leading to the identification of 10 compounds able to bind MRG15 with affinities ranging from 37.8 nM to 59.1 µM.[4]

In conclusion, MST resulted a robust and fast method for the identification of new ligands of MRG15.

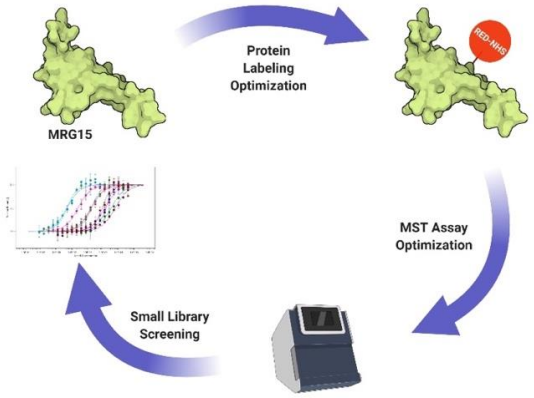


**Figure 1.** Workflow for the optimization and validation of the MST method.

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PO-041

# DEVELOPMENT OF 4,6-SUBSTITUTED-1,3,5-TRIAZIN-2(1*H*)-ONES AS CATALYTIC INHIBITORS OF THE HUMAN DNA TOPOISOMERASE IIA

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Human DNA topoisomerase IIα (htIIα) is one of the established targets in cancer chemotherapy. It catalyzes topological changes of the DNA molecule that are essential for the progression of transcription and translation, and its levels are higher in rapidly dividing cells[1]. There are several ways to tackle this established target, and agents targeting htIIα are divided into two broad groups. Of the established group of topo II poisons, a few molecules are routinely used in clinical practice. Due to the frequent occurrence of serious side effects, especially cardiotoxicity and induction of secondary tumors, as well as the observed resistance to topo II poisons, further drug development efforts have been undertaken to take advantage of other inhibition paradigms within the catalytic htIIα cycle. This has led to the development of catalytic inhibitors of htIIα that offer new opportunities to revisit this established target and inhibit it via alternative inhibitory mechanisms. Such molecules could, in principle, possess an improved safety profile with comparable anticancer efficacy[2]. In our previous research, 4,6-substituted 1,3,5-triazin-2(1*H*)-ones were discovered as monocyclic catalytic inhibitors of htIIα targeting its ATP binding site[3,4].

In order to improve the inhibitory activity and optimize the physicochemical properties of this class of compounds, development continued based on the previously synthesized active compounds. We retained the most promising substituted benzyl fragments at the position 4 of the triazin-2(1*H*)-one scaffold and introduced new bicyclic substituents at the substitution site 6. These substituents were selected from an analogous series of purine-based catalytic htIIα inhibitors[5]. After extensive molecular simulations examining the molecular recognition between the substituted triazin-2(1*H*)-one and purine inhibitors and the htIIα ATP binding site, several target compounds were synthesized and experimentally screened for htIIα inhibition. The optimized series showed improved inhibition of topo IIα compared to the initial series, and the catalytic mode of inhibition was confirmed for the selected active compounds along with binding to the isolated ATPase domain. The developed series represents a new step in the development of 4,6-substituted 1,3,5-triazin-2(1*H*)-ones towards potentially safer chemotherapies that exploit the paradigm of catalytic htIIα inhibition.

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PO-042

# OPPORTUNITIES FOR ORTHOSTERIC AND ALLOSTERIC DUAL TARGETING OF THE CANNABINOID RECEPTOR TYPE 1 AS POTENTIAL NEUROPROTECTIVE AGENTS

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The major players of the endocannabinoid system (ECS), the cannabinoid receptors CB1R and CB2R, form a multi-facetted therapeutic platform for managing several neuroinflammatory disorders. Whilst CB1R appears in high densities among neurons, regulating neurotransmitter release and synaptic strength, CB2R is found mostly in glial cells and microglia, modifying the ratio between pro- and anti-inflammatory cytokines released by these cells. A large volume of data indicates that compounds activating cannabinoid receptor type 1 (CB1R) can protect against neuronal damage produced by a variety of neuronal "insults" by limiting the release of glutamate, a key mediator in excitotoxic damage of neuronal cells and oligodendrocytes. In fact, an excessive glutamate release may involve toxic accumulation of calcium, resulting in excitotoxicity, a process common to many brain disorders that often leads to neuronal death.[1]
Our group already investigated the ability of the non-specific CB1R/CB2R orthosteric agonist FM-6b (Figure 1) to inhibit the glutamate release by activating CB1R. FM-6b was an effective CB1R agonist at presynaptic CB1R, controlling glutamate release, besides behaving as an agonist at the microglial CB2R.[2] More recently, we reported for the first time that in the presence of the pure CB1R Positive Allosteric Modulator (PAM) GAT229, known as the S-(-) enantiomer of the derivative GAT211, the glutamate release inhibitory effect of FM-6b was enhanced, and an inhibitory action on the release was revealed at low FM-6b concentrations, which were *per se* ineffective at the CB1R.[3]
The combination of orthosteric and allosteric ligands at CB1R, therefore, might be used to effectively inhibit the release of glutamate by promoting the activation of neuroprotective CB1R and, in addition, limiting the psychoactive side effects frequently associated with CB1R direct activation thanks to the presence of the allosteric ligand. Considering this evidence, we decided to translate this concept by developing a new series of compounds, namely **RF**, potentially able to bind to both allosteric and orthosteric sites at CB1R simultaneously. **RF** ligands were created by linking the pharmacophoric portion of the PAM GAT211 with that of the orthosteric agonist FM-6b (Figure 1). Besides the synthesis, we hereby describe the preliminary functional examination of this series of orthosteric-allosteric dual ligands.

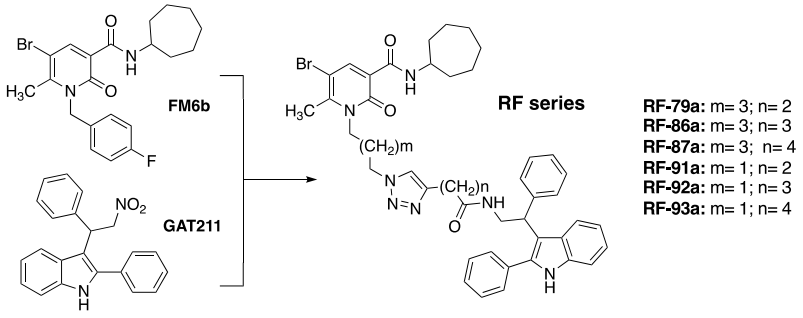


**Figure 1.** General structure of **FM6b**, **GAT211** and compounds belonging to the two **RF** series.

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PO-043

# DESIGN AND SYNTHESIS OF PYRIMIDINE COMPOUNDS AS NEW DISRUPTORS OF THE AURKA/N-MYC INTERACTION

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The enzyme Aurora kinase A (AURKA) is involved in centrosomes maturation and separation. In neuroblastoma, it displays a critical function independently of its kinase activity by binding to the oncoprotein N-Myc, preventing its degradation. This interaction contributes to the progression of neuroblastoma due to the interference with the cell-cycle exit of neuroblasts. While intense efforts have been made to develop AURKA inhibitors, the very low druggability of N-Myc has prevented any successful strategy for chemotherapeutic intervention. However, recent studies demonstrating the interaction between AURKA and N-Myc have provided an innovative therapeutic approach. These findings have prompted the identification of new compounds able to disrupt the AURKA/N-Myc complex, in order to promote N-Myc degradation. New studies indicated that some already known AURKA inhibitors were capable of blocking this protein-protein interaction[1]. **RPM1722** has emerged as a promising orthosteric bis-anilinopyrimidine AURKA inhibitor. Its bromine atom of the benzene ring is thought to be responsible for induced-dipole forces that freezes the enzyme in an unusual "DFG-out/loop-in" conformation. However, although in vitro assays **RPM1722** was able to also inhibit the N-Myc/AURKA interaction, it seemed quite ineffective in cell-based assays. This is probably because the greater speed whereby the more stable complex is formed removes free AURKA available to be bound by inhibitors. In this scenario, we described the design and synthesis of a new library of 2,4-disubstitued pyrimidine structurally related to **RPM1722**[2]. The pyrimidine core and the 4-carboxyphenylamino substituent in position 2 have been kept. Conversely, the nitrogen atom was replaced with an oxygen or sulfur and the phenyl ring in position 4 of the pyrimidine was modified by introducing halogens in 2, 3 and 4 positions. On the other hand, it has been also shown a covalent Coenzyme A inhibition of AURKA as a result of the formation of a sulfur bridge between the thiol group of the pantetheine tail and the side chain of Cys290 residue located in the activation loop of AURKA[3]. This mechanism is specific and provides the proof of concept for a potential irreversible inhibitory mechanism of AURKA which would possibly keep the AURKA in a conformation unsuitable for N-Myc binding. Hence, we retained the 2-(4-carboxyphenylamino) pyrimidine core of **RPM1722** as anchor site and decided to design a long branch with a terminal electrophilic warhead to mimic the pantetheine tail of acetyl-CoA to reach for the Cys290 residue.
All the newly synthetized compounds were tested *in vitro* to assess their ability to inhibit AURKA and most of them were found active in the nanomolar range. The putative covalent inhibitor proved to effective in the kinase inhibition assay with an $IC_{50}$ of 730 nM. The X-ray crystal structure of the most potent inhibitors were made to corroborate the biological results. Further investigations are needed to better understand the mechanism of action of our compounds.

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PO-044

# SYNTHESIS, DOCKING STUDIES AND PHARMACOLOGICAL EVALUATION OF SEROTONINERGIC LIGANDS CONTAINING A 5-NORBORNENE-2-CARBOXAMIDE NUCLEUS

Fiorino, F.;[a] Magli, E.;[a] Andreozzi, G.;[a] Morelli, E.;[a] Albrizio, S.;[a] Kędzierska, E.;[b] Bielenica, A.;[c] Kaczorc, A.A;[d,e] Perissutti, E.[a] and Caliendo, G.[a]

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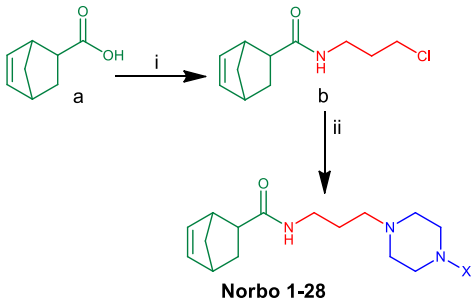
Pharmacological regulation of the 5-HT system has a great therapeutic potential, and therefore it is the subject of intense studies.[1] One of the most interesting 5-HT receptors is the 5-$HT_{1A}$ receptor, involved in a wide range of psychiatric disorders, and even in the proliferation of human tumor cells (PC3). 5-$HT_{2A}$ receptor stimulates the secretion of various hormones and mediates several processes such as intestinal motility and secretions, whereas 5-$HT_{2C}$ is probably the one with the most widespread distribution in the CNS and can mediate the action of 5-HT in virtually all brain areas. In this study we report the synthesis of two series of compounds, embodying 5-norbornene-2-carboxamide nucleus, that have been evaluated for their binding to the 5-$HT_{1A}$, 5-$HT_{2A}$, and 5-$HT_{2C}$ receptors. The designed molecules have been prepared following the procedure depicted in scheme 1. The combination of structural elements (heterocyclic nucleus, propyl chain, and 4-substituted piperazine) known to be critical for affinity to 5-$HT_{1A}$ receptors and the proper selection of substituents led to compounds with high specificity and affinity toward serotoninergic receptors. Moreover, the compounds displaying better affinity and selectivity binding profiles were selected in order to be tested by *in vitro* and *in vivo* assays to determine their functional activity. Besides the outstanding 5-$HT_{2A}$ receptor affinity and selectivity of **Norbo-14** supporting a 3,4 dichlorophenyl group (*Ki* = 17.93 nM) and **Norbo-18** supporting a 4-fluorophenyl group (*Ki* = 18.65 nM) other interesting *Ki* values were those of compounds **Norbo-20,** supporting a 3-trifluoromethylphenyl group (*Ki* = 22.86 nM with), and **Norbo-3,** supporting a 2,3-dimethyphenyl group (*Ki* = 23.49 nM). When comparing the evaluated series of derivatives to 5-$HT_{2C}$ receptor selective ligands, as RS-102221, one can point norbornene derivatives supporting the 2-chloro (**Norbo-10**) and 2-fluorophenylpiperazine (**Norbo-15**) moieties as terminal group are the most promising, with their Ki values of 13 and 31nM, respectively. None of the tested compounds expressed significant antagonistic activity against the $D_2$ receptor. **Norbo 8**, supporting a 4-methoxyphenyl group, showed an agonist profile ($pEC_{50}$ =6.78 ± 0.09; $E_{max}$ (%) =167 ± 2.3) towards the receptors. Finally, to rationalize the different binding affinities/activities, molecular docking studies were carried out on the complete series of derivatives. The obtained results further support the choice of the 5-norbornene-2-carboxamide nucleus for the preparation of serotoninergic ligands endowed with 5-$HT_{1A,}$ 5-$HT_{2A}$ and 5-$HT_{2C}$ affinity and activity.




**Norbo 1-28**

Scheme 1: (i) $Cl(CH_2)_3NH_2$·HCl, DCC, HOBt, TEA, $CH_3CN$, r.t., 24h; (ii) 4-X-substituted- piperazine, $K_2CO_3$, NaI, $CH_3CN$, 70°C, 24 h

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PO-045

# DEVELOPMENT OF A LIGAND-BASED APPROACH FOR THE IDENTIFICATION OF NEW HEME OXYGENASE-1 INHIBITORS

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Heme oxygenase-1 (HO-1) is a stress-inducible enzyme that exerts an active role in maintaining cellular homeostasis in response to oxidative stress. On the other hand, up-regulation of the HO-1 gene (*HMOX1*) has been linked to tumor aggressiveness and drug resistance phenomena in a cancer cell-specific manner. Accordingly, *HMOX1* gene silencing or HO-1 pharmacological inhibition with both competitive and non-competitive inhibitors proved to be an effective strategy to enhance chemotherapy efficacy in different tumor cell lines.[1]

To identify novel potent non-competitive HO-1 inhibitors, we have recently used a structure-guided fragment-based approach to design and synthesize the first set of hit molecules.[2] Moreover, to improve our previously reported 3D-QSAR model,[3] and refine it based on our in-house library of azole-based compounds, we created an updated version of the old model. The new 3D-QSAR model has been tested and compared to the previous one, and it showed improvement in predicting the HO-1 inhibitory activity. Subsequently, the new model has been used to generate novel HO-1 inhibitors through growing and scaffold hopping experiments, and the results are herein reported.

These findings support ours *in silico* approach as a successful strategy to rapidly and efficiently drive the identification of new hit compounds for anticancer *in vitro* evaluation and validate the use of our updated 3D-QSAR model for the first time. The preliminary antiproliferative activity of the most promising compound toward selected cancer cell lines is in progress and will be reported in due time.

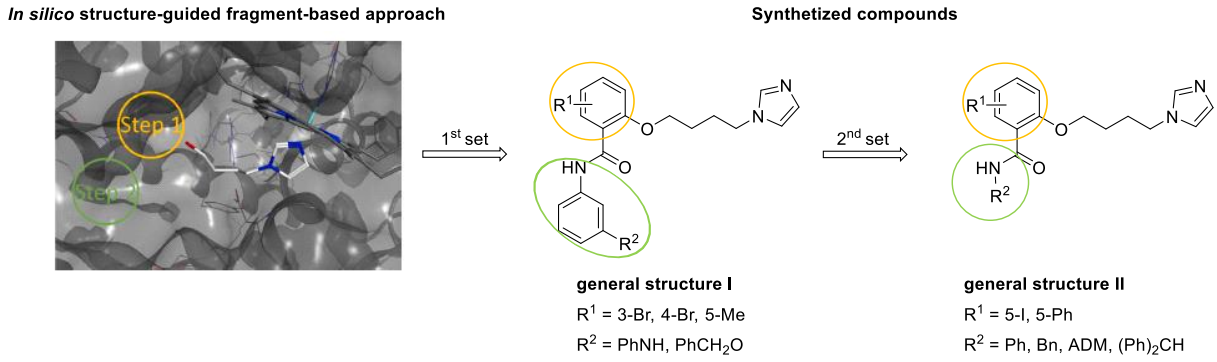


**Figure 1.** Two steps ligand growing approach and general structure of newly synthesized imidazole-based compounds.

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PO-046

# BIOISOSTERIC REPLACEMENT OF THE UREA FUNCTION IN FPR2 AGONISTS WITH NEUROPROTECTIVE PROPERTIES

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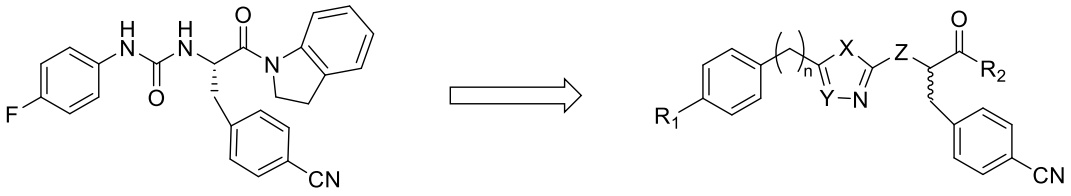
The resolution of inflammation is a physiological process that leads to the restoration of normal function in tissues damaged by internal or external insults. Chronic or unresolved inflammation is a key pathological hallmark in various diseases, including neurodegenerative disorders. The resolution of inflammation is finely orchestrated through the release of endogenous mediators, known as Specialized Pro Resolving Mediators (SPMs), that induce a switch from the production of pro-inflammatory to anti-inflammatory mediators. SPMs elicit their effects through the interaction with specific membrane receptors, including the Formyl Peptide Receptor 2 (FPR2), which represent valuable targets for developing new therapeutic approaches for the treatment of chronic inflammation.[1]

We recently identified a class of potent non-peptidic FPR2 agonists with a ureidopropanamide scaffold having neuroprotective, anti-inflammatory, and pro-resolving activities in several in vitro and in vivo models of Central Nervous System (CNS) disorders characterized by chronic neuroinflammation.[2,3] Despite the encouraging pharmacodynamic properties, the ureido function raises issues related to water solubility and potential toxicity. Therefore, the urea function has been isosterically replaced with different heteroaromatic rings. We report here on the rational design, synthesis, and preliminary evaluation, supported by dockings to FPR2, of the observed activity of this new set of compounds.




**AMS-21**
FPR2 EC50 μM (efficacy, %): 0.026 μM (140 %)
Metabolic stability (t1/2, min): 21 min

$R_1$: F; Br; n: 0-1; X: O; S; Y: N; C; Z: NH; $CH_2$;
$R_2$: indolin-1-yl; 6-F-indolin-1-yl

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PO-047

# INTERACTION STUDIES OF NEW BIOACTIVE MACROLIDE DERIVATIVES WITH BACTERIAL RIBOSOME

Jednačak, T.;[a] Mikulandra, I.;[a] Kapustić, M.;[a] Kušec, I.;[a] Smokrović, K.;[a] Hošnjak, E.;[a] Sanemit, S.;[a] Piantanida, I.;[b] Košćak, M.;[b] Bertoša, B.;[a] Barišić, A.;[a] Zangger, K.[c] and Novak, P.[c,]

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Owing to their high efficacy and safety, macrolides have been in widespread clinical use for the treatment of upper and lower respiratory tract infections.[1,2] The reversible binding of macrolides to the 23S rRNA of the large ribosomal subunit, at or near the peptidyl transferase center, blocks the exiting tunnel for newly synthesized peptides and thus inhibits the synthesis of bacterial proteins. The chemical structure of clinically relevant macrolides is characterized by a macrolactone ring, usually consisting of 14–16 atoms, substituted by polar and non-polar groups and linked to one or more carbohydrate units via glycosidic bonds. Despite the fact that new antibiotics have been developed, some bacteria have acquired broad resistance, representing a global medical problem which can only be resolved by the discovery of new and more potent drugs. Our previous studies have shown that linking known macrolide antibiotics to bioactive thiosemicarbazones resulted with novel conjugates, the macrozones, efficient against multidrug-resistant strains.[3,4] It has also been reported that some thiosemicarbazones and their metal complexes possess anti-infective, anti-tumor and anti-inflammatory activities.[5]

In this work, we studied the interactions of new bioactive macrolide conjugates and their metal complexes with the *Escherichia coli* ribosome by a combined use of NMR spectroscopy, fluorescence measurements and molecular modelling simulations. Saturation transfer difference (STD), WaterLogsy and transferred nuclear Overhauser effect (trNOE) NMR experiments provided valuable data about ligand conformations and binding epitopes. Fluorescence spectra were further processed by multivariate data analysis methods to assess binding constants. Molecular modelling simulations revealed the macrolide binding sites at the *E. coli* ribosome.

The obtained results can further be exploited in the process of designing novel macrolide derivatives with activity against resistant pathogens.

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PO-048

# DESIGN, SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF MOLECULAR HYBRIDS BETWEEN CORTICOSTEROIDS OR RETINOIDS WITH $H_2S$ DONORS FOR PSORIASIS TREATMENT

Giordano, F.;[a] Sparaco, R.;[a] Santagada, V.;[a] Caliendo,G.;[a] Citi, V.;[b] Calderone, V.;[b] Muscarà, M. N.;[c] Costa S.K.P.;[c] Rimoli, M. G.;[a] Morelli , E.;[a] Corvino, A.;[a] Frecentese, F.[a]

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Psoriasis is a chronic and relapsing immune-mediated inflammatory skin disease, characterized by uncontrolled keratinocyte proliferation and dysfunctional differentiation.[1] Histological features of psoriasis plaques are acanthosis (epidermal hyperplasia), increased vascularity in the dermis, and inflammatory infiltrate.[2] Since $H_2S$ has proven to be involved in inflammation, pruritus and cytoprotection,[3] molecular hybrids have been developed for merging the beneficial effect of $H_2S$ to pharmacological effect of the starting drug. Our research group has indeed recently focused its attention on the hybridization of pharmacophoric units from different bioactive compounds developing new chemical entities (by direct linkage, spacer linkage, fusing the moieties, etc) able to retain the pre-selected characteristics of the original templates and exerting improved pharmacological activity and/or reduced toxicity.[4]

The project has been aimed to the synthesis of molecular hybrids containing an antipsoriasis drug, represented by glucocorticoids or retinoids that have been coupled with different $H_2S$ donors such as 4-hydroxybenzothioamide (TBZ), 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH), S-ethyl 4-hydroxybenzodithioate (HBTA) and 4-hydroxyphenyl isothiocyanate (HPI). Betamethasone 17-valerate and triamcinolone acetonide have been selected as glucocorticoids, while acitretin and tazarotenic acid were selected as retinoids: four series of molecular hybrids (compounds **I-XVI**) have been synthesized. Microwave assisted synthesis has been adopted when opportune for a significant reduction in reaction times that is associated with increased yields and improved purity of products obtained reducing the use of solvents and resolving agents in the work-up of reaction. Due to the involvement of proinflammatory mediators at different stages of the psoriasis, herein we describe the anti-inflammatory activity of the $H_2S$-releasing glucocorticoids, that has been evaluated through the quantification of TNFα, IL-6 and IL-1 beta by using ELISA sandwich assay. Moreover, two selected molecular hybrids, corresponding to compounds **V** and **VIII** derived from triamcinolone acetonide, were tested *in vivo* in a murine model of psoriasis.

*In vitro* Elisa assay demonstrated that betamethasone-17-valerate and its derivatives were not effective in reducing TNF levels, while triamcinolone acetonide and its derivatives limited TNF production and were the most promising compounds. Triamcinolone acetonide-TBZ (**V**) and triamcinolone acetonide-HPI (**VI**) were the most active. Similar results were found for the determination of IL-6 and IL-1 beta levels.

Based on *in vitro* results, two molecular hybrids, **V** and **VIII** were selected for *in vivo* evaluation: psoriasis was induced by topical administration of imiquimod cream (IMQ) on mice dorsal skin for 5 consecutive days. Mice were then topically treated in the dorsal skin with 65 mg of a nanodispersion (DLC) alone or DLC containing 1% of selected molecular hybrids or 1% of triamcinolone acetonide. The determination of some Psoriasis Area Severity Index (PASI), skin thickness and itch behavior were evaluated daily. In the last day of evaluation ($6^{th}$), treatment with 1% of **V** or **VIII** based nanodispersion, rather than triamcinolone acetonide itself, was able to reduce most of psoriasis scores. Pharmacological tests aimed to the assessment of the synergic/complementary effect derived by the release of $H_2S$ and of the retinoids are ongoing.

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PO-049

# MDM2/4 HETERODIMER INHIBITORS AND 5-FU AS NOVEL NANPARTICLE-MEDIATED COMBINATION FOR CANCER FIGTHING

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The nucleolus is a subnuclear structure whose primary function is the ribosome biogenesis. Emerging evidence suggest that some chemotherapy drugs as 5-Fluorouracil (5-FU), commonly used for different tumors, can perturbate ribosome biogenesis causing a condition known as nucleolar stress. In this condition, some ribosomal proteins bind to murine double minute 2 (MDM2), the E3 ubiquitin ligase involved in p53 degradation, and inhibit MDM2 suppression of p53 activity. Thus, high levels of p53 result in cell cycle arrest and/or apoptosis[1]. Nevertheless, the majority of tumors escape death maintaining low level of p53 by hyperactivating MDM2 and MDM4. In this context, it seems reasonable to improve the efficacy of current chemotherapy and overcome drug resistance by exploiting a new therapeutic strategy consisting in the combination of 5-FU with MDM2/4 inhibitors. Recent evidence indicates that MDM2/MDM4 heterodimers produce a more effective inhibition of p53 than MDM2 homodimers in terms of ubiquitination, degradation and p53 transactivation activity. Consequently, a more efficient p53 reactivation could be achieved targeting the MDM2 and MDM4 interface. So far, only a dodecapeptide that overlaps the MDM4 COOH-terminus domain, named Pep3, was reported as MDM2/4 disruptor[2]. Nevertheless, the size of this peptide did not allow defining the minimal structural requirements responsible for the activity, a prerequisite to obtain small organic compounds or low MW peptide ligands, usually considered more "drug-like" candidates. On this premises, a library of smaller peptides able to disrupt the MDM2/4 heterodimer have been designed and developed, with two of them, VLP-13 and VLP-24, tenfold more potent than Pep3[3]. In order to overcome the high susceptibility of the peptides to *in vivo* degradation, they have been loaded, alone or together with 5-FU, into specific nanoparticle (NPs) delivery-system, that have proved to be effective in activating the nucleolar stress pathway and reducing cell proliferation on colon cancer cells (see Figure 1).

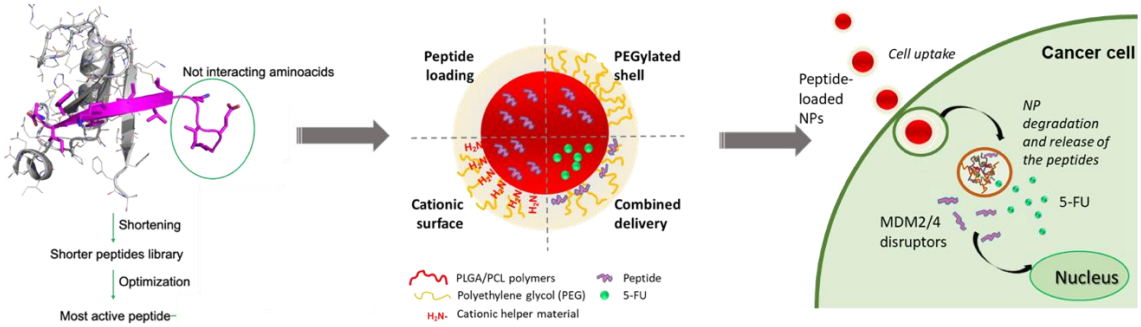


**Figure 1**

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PO-050

# DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DUALSTERIC CANNABINOID RECEPTOR TYPE 2 (CB2R) LIGANDS

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The discovery of biased agonism has had important implications for the understanding of GPCR biology, revolutionizing the traditional two state receptor model with a more dynamic vision of the receptors as proteins able to switch among different states each responsible of specific functional outcomes. One of the most promising strategies leading to signalling-selective (biased) GPCR modulators is represented by dualsteric compounds, consisting of allosteric and orthosteric pharmacophores combined in one single ligand through particular spacers. These derivatives might show a GPCR modulation with a unique receptor-subtype and signaling selectivity profile, fewer side effects and resistance compared to monovalent compounds. We focused our study on cannabinoid receptor 2 (CB2R) synthesizing two new classes of dualsteric ligands, namely **JR**, linking the pharmacophoric portion of the CB2R positive allosteric modulator (PAM) **EC21a**[1] with that of **LV62**[2], a CB2R selective orthosteric agonist, with different spacers. The two classes differ for the attaching position of the linker at the level of the orhosteric portion (Figure 1).

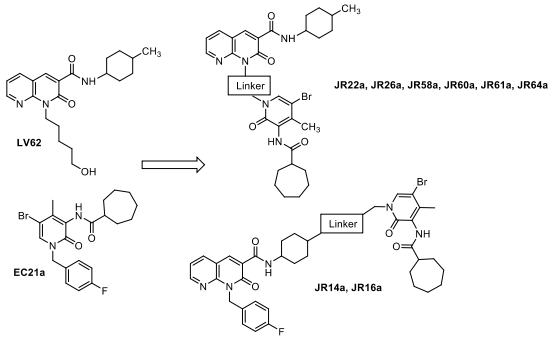


**Figure 1.** General structure of **LV62**, **EC21a** and compounds belonging to the two **JR** series.

Functional examination highlighted, for the majority of compounds, a significant signaling 'bias' in favor of G protein activation over βarrestin recruitment. Moreover, biological analysis showed a modulation of the interleukins release in human microglial cell line (HMC3) exposed to LPS/TNFα stimulation without any relevant cytotoxic effect. These results indicate an ability to contrast inflammatory processes in microglial cells, counteracting a mechanism that supports the onset, progression and severe symptomatology of several neurodegenerative diseases.

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PO-051

# TACKLING ANTIMICROBIAL RESISTANCE WITH PLANT NATURAL COMPOUNDS TARGETING LSRK

Linciano, P.;[a] Cavalloro, V.;[b] Pietrocola, G.;[c] Motta, C.;[c] Rossi, D.;[a] Rossino, G.;[a] Listro, R.;[a] Martino, E.;[b] Collina, S.[a]

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Beside COVID-19, another pandemic is underway in the world but, despite the warnings of the WHO, it does not arouse the attention it should: the spread of bacteria resistant to one, several or all the antibiotics available to kill them. Antimicrobial resistance (AMR) is expected to become the first cause of death by 2050, thus the search for new targets and innovative antimicrobials has become mandatory. A promising antibacterial strategy to deal with the development of AMR aims to interfere with bacterial quorum sensing (QS). The QS is the most effective cell-to-cell mechanism that bacteria use to communicate, coordinate and act as a population, thereby gaining some benefits that otherwise were unattainable at planktonic state. The "words" used by bacteria to communicate are small endogenous molecules called autoinducers (AIs). One of the key bacterial enzymes that triggers and stokes the AI-2 mediated QS cascade, in both Gram+ and Gram-, is the kinase LsrK. Thus, the exploitation of LsrK as a new target for the development of novel QS quenching drugs may represents a huge opportunity in fighting AMR. Because the LsrK pathway has been discovered quite recently, this target is still poorly explored from a pharmaceutical standpoint; thus, searching for new and developable synthetic LsrK inhibitors is challenging.[1] Nature-aided drug discovery is a successful in strategy in the finding bioactive compounds. Accordingly, we recently performed a combined Ligand-Based and Structure-Based Virtual Screening (VS) on an in-house library of plant secondary metabolites. As a result, three natural compounds were proposed, namely HibA, HibB, and ASME. They were isolated in milligram scale and in high purity from the proper natural sources.[3] The binding of HibA, HibB and ASME at the purified LsrK was evaluated using two complementary spectrophotometric approaches: circular dichroism (CD) and fluorescence spectroscopy (FS). The accordance between the results obtained by CD and FS validates the binding of both molecules at LsrK, with a $K_D$ in the sub-micromolar range, and consequently the computational model set up for the VS. Besides, a suitable synthetic strategy for the in-house preparation of the LsrK substrate (DPD) was developed. This is not to be taken for granted as DPD is not purchasable and it is only available through high-cost custom synthesis; at the present, the unavailability of DPD greatly limited the research around LsrK. With the substrate DPD in our hands, HibA and HibB were assessed for their LsrK inhibitor activity resulting in an $IC_{50}$ in the low µM range. Accordingly, further in vitro investigations to evaluate the capability of the two compounds to interfere with QS and to restore the susceptibility of resistant bacterial strain to antimicrobials are ongoing. Altogether, the adopted strategy may lead to novel antibacterial compounds with new modes of action, thus providing a paradigm shift in fighting AMR.

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PO-052

# EXPLAINABLE MACHINE LEARNING AND FEATURES ANALYSIS FOR DIFFERENTIATING SINGLE OR MULTI-TARGET ACTIVITY AGAINST THE CANNABINOID SYSTEM

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The endocannabinoid system including the CB1 and CB2 receptor isoforms has been demonstrated to play a pivotal role in several pathological pathways. It has been implicated in several pathologies including neurodegenerative diseases, cancer, neuropathic and inflammatory pain, obesity, and inflammatory bowel disease. given the high similarity of CB1 and CB2, finding selective ligands and rationalizing potential selectivity-conferring features are difficult tasks. In the study presented herein, machine learning has been used to predict compounds with dual- or single target CB1/2 activity [1]. For model derivation, CB1/2 compound activity data was extracted from the latest release of the ChEMBL database[2], and different 2D molecular fingerprints were used as descriptors. Finally, Shapley values[3] were computed to identify features determining correct predictions and explain machine learning models. The newly developed models are expected to aid in the identification of new active compounds for CB1 and/or CB2 or repurposing of known actives.

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PO-053

# SYNTHESIS, ANTIPROLIFERATIVE AND ANTIOXIDANT PROPERTIES OF HIGHLY FUNCTIONALIZED PYRAZOLES

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Compounds **1** (Figure 1) showed promising antioxidant activity by inhibiting ROS formation in platelet.[1] The substitution of the catechol moiety (X group, Figure 1) modulates the ability of the compounds to block ROS formation. To further extend the structure-activity relationships (SARs) and evaluate the insertion of an additional substituent on the pyrazole ring, derivatives **2** and **3** were designed, synthesized and characterized. The adopted synthetic strategy involved a divergent approach starting from the common intermediates **A** (Figure 1). The synthesis of pyrazoles **A** was carried out by a sequential, one-pot procedure which represents a cheaper alternative either in terms of time or cost.[2] The derivatization of either the ester or the amino functionalities led to the formation of derivatives **2** and **3**, respectively.

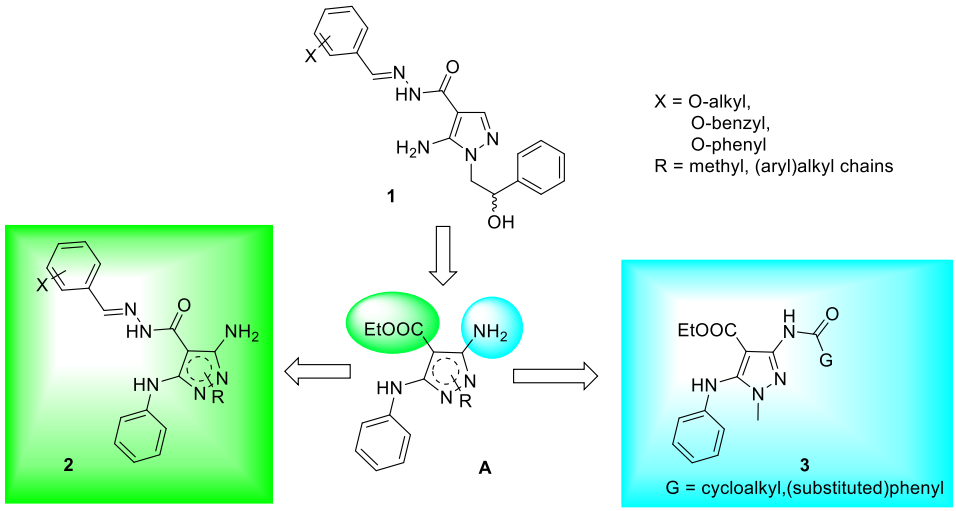


**Figure 1,** general structures of synthesized compounds.

The obtained derivatives were screened for their antiproliferative activity in cell-based assay on a panel of tumour and normal cell lines. Additionally, the compounds were tested on human platelets to evaluate their properties against ROS formation and aggregation. The synthetic procedure, the structural assignments as well as the biological properties of the prepared pyrazoles will be presented and discussed in the presentation.

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PO-054

# RATIONAL DESIGN AND SYNTHESIS OF POTENTIAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT) RADIOLIGANDS FOR IN VIVO IMAGING OF P2X7 RECEPTORS

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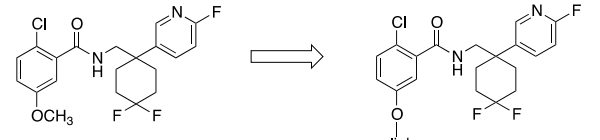
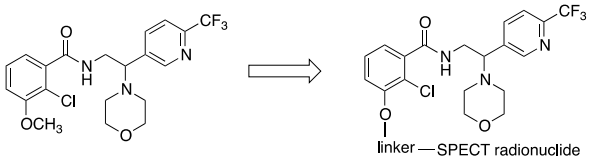
The purinergic P2X7 receptor (P2X7R) is an ion gated channel activated by ATP and is involved in microglial activation, proliferation, and apoptosis. Under physiological conditions, P2X7Rs expression in the CNS is rather limited. Following a brain injury, the expression of P2X7Rs increases substantially, thereby promoting inflammasome formation and the release of proinflammatory cytokines/chemokines.[1] Several studies have highlighted a link between P2X7R expression and neurodegenerative disorders, such as sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. For this reason, P2X7R has been proposed as a target for in vivo imaging of neuroinflammation as an alternative to the translocator protein (TSPO). Several P2X7R antagonists have been radiolabeled and studied during the last decade as potential PET radioligands, whereas no SPECT radioligand has been developed so far.[2] SPECT radioligands offer several advantages over PET radioligands including longer half-life and ease of radiosynthesis.[3]
From a literature survey, we identified compound 1 and compound 2 as a valuable starting point for developing a potential SPECT radioligand for the in vivo imaging of P2X7R. In fact, both compounds had inhibitory activity in the nanomolar range and have functional groups amenable for further functionalization with a linker bearing the SPECT radionuclide. A preliminary docking study on compounds 1 and 2 evidenced that the benzamide moiety is oriented towards the extracellular part of the binding site. Therefore, the methoxy group on the benzamide moiety will be the anchoring point of the linker bearing the SPECT radionuclide. Different linker lengths will be studied, and technetium-99m (Tc-99m) will be selected as SPECT radionuclide.
We will report the rational design and the synthesis of the potential SPECT P2X7R radioligands.




Compound 1
P2X7R $IC_{50}$= 0.83 nM




Compound 2
P2X7R $IC_{50}$= 3.5 nM

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PO-055

# NEW AMIDINE-BENZENESULFONAMIDES AS iNOS INHIBITORS FOR THE THERAPY OF THE TRIPLE BREAST CANCER

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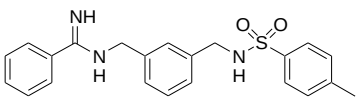
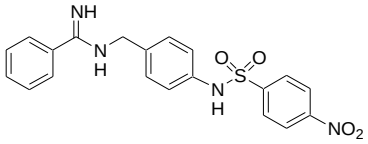
Triple negative breast cancer (TNBC) is a specific breast cancer subtype, and poor prognosis is associated to this tumour when it is in the metastatic form.[1] The overexpression of the inducible Nitric Oxide Synthase (iNOS) is considered a predictor of poor outcome in TNBC patients, and this enzyme is reported as a valuable molecular target to compromise TNBC progression.[2]
As part of an ongoing project on the development of new iNOS inhibitors able to counteract cancer progression,[3,4] new amidines containing a benzenesulfonamide group were designed and synthesized as selective iNOS inhibitors. An in vitro biological evaluation was performed to assess compounds activity against both the inducible and constitutive NOSs. The most interesting compounds **1b** and **2b** (Figure 1**)** were evaluated on MDA-MB-231 cells as antiproliferative agents, and **1b** capability to counteract cell migration was also studied. Finally, an in-depth docking study was performed to shed light on the observed potency and selectivity of action of the most promising compounds.




**1b**
**i**NOS $IC_{50}$=0.065 μM
eNOS/iNOS selectivity >770 folds




**2b**
**i**NOS $IC_{50}$=0.832 μM
eNOS/iNOS selectivity >60 folds

**Figure 1.** Chemical structure of the most active benzensolfonamide derivatives **1b** and **2b**.

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PO-056

# SCREENING WORKFLOW FOR PROBING THE BINDING OF SMALL MOLECULES TO PHOSPHODIESTERASE 9 BY MASS SPECTROMETRY

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Paralleling life expectancy increase, neurodegenerative diseases are predicted by World Health Organization to become the second most prevalent cause of death by 2040.[1] Worldwide, 44 million people are affected by dementia, and current treatments are mostly limited to symptoms management rather than providing an effect on the underlying biochemical mechanisms triggering disease onset and progression.[2] Thus, the need for the identification of novel targets and new therapies overcoming limited efficacy and adverse effects of approved drugs emerges.[3]

The growing interest on the therapeutic potential of natural compounds against neurodegeneration is often paralleled by a limited understanding of the undergoing biochemical pathways in which these molecules may be involved. Although, computational tools and efficient cell-free ligand-target interaction studies are nowadays enrolled and combined in the drug discovery and/or repurposing workflow.

Importantly, neuroprotection and cognitive enhancement are associated with increased 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) levels in brain, resulting from the inhibition of phosphodiesterases (PDEs). In particular, PDE5 and PDE9 are gaining attention as potential targets to modulate signal transduction by regulating cAMP and/or cGMP, thus mediating neurotransmitter release, amelioration of microvascular dysfunction and neuronal plasticity.[4]

In this contribution, we describe a screening workflow for the identification, or the re-discovery, of compounds targeting PDE9. The features of this isoform, including its expression in the brain, the presence within its structure of a peculiar accessory pocket, the asymmetry between the two subunits composing the dimer, and the selectivity for chiral species, prompt the development of neuroprotective agents targeting this enzyme.[5]

The evaluation of PDE inhibitors through commercial screening kits requires the use of a luminescence reader and such tests are often not scalable and expensive. In this contribution, we will describe the development of a cell-free ligand screening protocol based on mass spectrometry, which allows to detect noncovalent ligand-target interactions rapidly and quantitatively thorough an inexpensive and potentially high-throughput workflow. In particular, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) techniques will be considered, and advantages and disadvantages of each will be dissected. We will report the preliminary screening towards PDE9 of a small panel of natural and semi-synthetic molecules, and according to the results of MS experiments, compounds will be ranked in terms of calculated binding affinity (BA) values. Moreover, collision-induced dissociation (CID) experiments will be used to investigate the relative gas-phase stability of the complexes. Taken together, this information allows accelerating hit identification and optimization, and the in *vitro* validation of preliminary data.

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PO-057

# CYCLOHEPTATHIOPHENE-3-CARBOXAMIDES AS INFLUENZA POLYMERASE PA-PB1 PROTEIN-PROTEIN INTERACTION INHIBITORS

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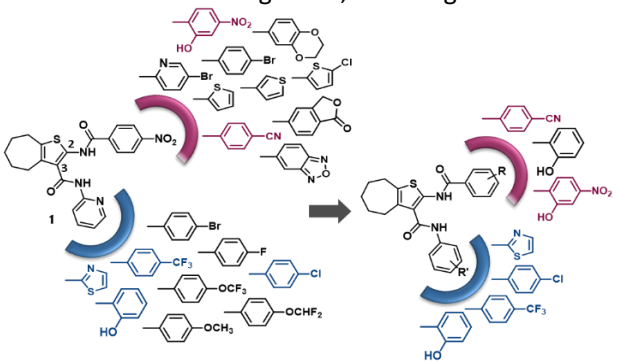
Viruses can infect humans through different interactions occurring between viral and host proteins, indicating that targeting these PPIs can represent a promising approach for identifying new antiviral agents. Influenza virus (flu) RNA-dependent RNA polymerase (RdRP) is an heterotrimer complex composed of PB1, PB2, and PA subunits, which extensively interact with each other and with multiple host factors, playing vital roles in virus replication, host adaptation, interspecies transmission, and pathogenicity. Inhibition of flu RdRP functions by PPI disruptors clearly represents a valid means to identify compounds endowed with a broad spectrum of action and a reduced propensity to develop drug-resistance, which are the main issues of antiviral drugs.[1,2] Among PPIs by RdRP subunits, our group focused on the development of PA-PB1 interface inhibitors. In particular, compounds based on the cycloheptathiophene-3-carboxamide (cHTC) scaffold showed the ability to disrupt PA-PB1 complex formation, to potently inhibit flu RdRP and viral growth, and a high barrier to drug resistance.[3-5]



Starting from the recently identified compound **1**,[4,5] in the present study, additional cHTC compounds were synthesized by alternately modifying the moieties at the C-2 and C-3 position of the cHTC core, but also combining the best moieties emerged into an additional set of analogues. The study led to identify new potent anti-flu compounds characterized by the ability to interfere with PA-PB1 association and to inhibit RdRP functions. In depth studies were performed to determine their activity against a panel of fluA and fluB strains and their ability to interfere with PA-PB1 heterodimerization in a cellular context. Finally, the pharmacokinetic profile was investigated to assess the drug-like properties of the new small molecules.

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PO-058

# COMBINING PHARMACOPHORE MODELING AND MOLECULAR DOCKING FOR THE VIRTUAL SCREENING OF TARGETED REACTIVE LIBRARIES AS POSSIBLE COVALENT RIPK1 INHIBITORS

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Receptor interacting protein kinase 1 (RIPK1) has emerged as a promising therapeutic target for the treatment of a wide range of human neurodegenerative, autoimmune, and inflammatory diseases. By modulating a type of programmed necrosis called necroptosis, RIPK1 was reported as a potential therapeutic target for ocular diseases, including glaucoma, dry age-related macular degeneration, and retinitis pigmentosa.[1] Non-covalent RIPK1 inhibitors have been developed and have demonstrated safety in preclinical models and clinical trials.[2] However, the development of covalent inhibitors could potentially increase target selectivity and residence time, thus reducing the dose and frequency of administration and, consequently, the risk of possible adverse effects. Indeed, covalent inhibitors have the advantage of very tight and irreversible binding, allowing the design of compounds with small molecular mass but with high potency.[3] Computational methods can be exploited to improve the selectivity of a reactive compound by both optimizing the non-covalent interactions with the binding site and tailoring the chemistry of reaction with a specific site of alkylation.[4]

The aim of this project is to identify and develop novel selective RIPK1 covalent inhibitors, combining pharmacophore- and structure-based virtual screening to target possible reactive residues identified through structural analysis of the available RIPK1 crystal structures.

We leveraged the Reaxys® database for mining the available literature to select 55 potent RIPK1 inhibitors, 44 of which having an $IC_{50}$ in the nanomolar range. 54 out of 55 compounds had a molecular weight between 300 and 550 and contained several aromatic moieties, together with at least one amide group. A structural similarity clustering based on Tanimoto similarity index was then performed, leading to the selection of 23 different compounds, which were then used to generate a 3D common feature pharmacophoric hypotheses, constituted by two hydrogen bond acceptors, a hydrophobic group, and an aromatic ring, as well as excluded volumes.

From the analysis of RIPK1 3D structure, lysine 45 was identified as a good target residue for covalent binding of ligands, as it is in the proximity of the inhibitors binding site, with good solvent accessibility. Sulphonyl fluoride exchange (SuFEx) compounds are known to react with lysine and tyrosine with high selectivity.[14] Thus, 989 SuFEx compounds with molecular weight between 300 and 550, and containing aromatic groups and at least an amide, were retrieved from the Reaxys® database.

After a preliminary pharmacophore-based virtual screening, 640 compounds were docked into the active site of RIPK1 (PDBID: 4itj) using the recently developed AutoDock Reactive Docking method, which allows prospective prediction of covalent binding sites on a protein through the simulation of the reaction event between the covalent residue and the warhead of the ligand.[4,5] The combined pharmacophore- and structure-based virtual screening used in this work allowed the identification of 6 possible covalent RIPK1 inhibitors, which are currently under *in vitro* validation.

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PO-059

# DEVELOPMENT OF A POTENT BICYCLIC PEPTIDE INHIBITOR OF HUMAN UROKINASE-TYPE PLASMINOGEN ACTIVATOR, A PROTEASE INVOLVED IN CANCER PROGRESSION

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Human urokinase-type plasminogen activator (huPA), a trypsin-like serine protease that participates in the turnover of extracellular matrix (ECM) proteins, is implicated in tumor growth and invasion[1] and inhibitors of huPA are being developed for therapy[2]. Herein we report the development of a novel bicyclic peptide inhibitor of huPA that is around 15-fold more potent than the best bicyclic peptide inhibitor previously isolated to this target [3] and crystallized the complex. This revealed an extended structure of the peptide with both peptide loops engaging the target to form a large interaction surface with multiple hydrogen bonds and complementary charge interactions, explaining the high affinity of the inhibitor. The interface resembles that between two proteins and suggests that such small highly constrained bicyclic peptides (<2 kD) have the potential to act as small therapeutic protein mimics.

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PO-o6o

# DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW *N*-ALKYL-4-OXO-1,4-DIHYDROQUINOLIN-3-ADAMANTILAMIDES DERIVATES AS FLUORESCENT PROBES FOR THE DETECTION OF CB2 RECEPTOR

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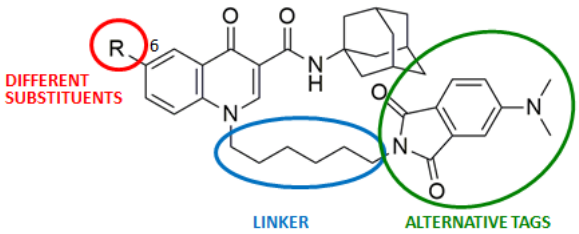
Cannabinoid receptor subtype 2 (CB2R), part of the endocannabinoid system (ECS), is overexpressed in the early stages of neuroinflammation and in the cancer onset and progression. To clarify the role of CB2R in these pathologies, several CB2R fluorescent ligands have been developed as "green" and safe diagnostic tools. Keeping this in mind, we recently developed as CB2R fluo-ligand, the compound **1** [1,2] (Figure 1), bearing a quinolone core (responsible for CB2R affinity and selectivity) linked, by an hexamethylene linker, to the 4-*N*,*N*-dimethylaminophthalimide (as green emitting tag). Compound **1** showed a good affinity towards CB2R ($K_i$ = 130 nM) and a very high selectivity, since it is devoid of affinity at CB1R subtype (14 %@ 1µM). In order to improve the pharmacodynamic properties of the lead compound **1**, we proceeded with its optimization by: i) the insertion of substituents on the quinolone core to improve CB2R affinity; ii) the modification of the length of the spacer linking the fluorescent tag to the quinolone scaffold; iii) the introduction of fluorescent tags (alternative to the phthalimide nucleus) with an emission spectrum shifted towards longer wavelengths (Red and NIR regions) to have greater versatility for other fluorescence techniques. The compounds resulting from this study were tested for their affinity and selectivity towards CB2R, as well as for their fluorescent properties. Molecular docking simulations complemented the experimental findings providing a molecular rationale behind the observed CB2R affinities, hence paving the way for a rational design of new and better performing fluorescent probes.




**1**

**Figure 1. Figure 1.** Lead compound **1**.

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PO-061

# TOWARDS THE DEVELOPMENT OF γ-LACTONE DERIVATIVES AS A NEW CLASS OF PROTEIN TYROSINE PHOSPHATASE B (MptpB) INHIBITORS FOR TB TREATMENT

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Despite the global effort to discover innovative treatments against tuberculosis (TB), this disease is still among the leading causes of mortality from a single infectious agent, surpassed in 2020 only by COVID-19.[1] *Mycobacterium tuberculosis* (Mtb) can secrete two Low-Molecular-Weight Phosphatases (LMW-PTPs), MptpA and MptpB, inside the macrophage's cytoplasm. These enzymes are essential for the pathogen *in vivo* viability within the host cells because they interfere with the immune response. Therefore, LMW-PTPs were validated as promising targets for the development of innovative anti-TB agents.[2]

Starting from a virtual screening campaign and subsequent structure elucidation studies guided by crystallographic analysis, the unexpected γ-lactone derivative **1** (Fig. 1) revealed a promising inhibitory activity on recombinant MptpB ($IC_{50}$ ≈ 48 μM).[3] SAR studies allowed to disclose several effective derivatives, whose docking poses were shown to interact within the enzyme active site through the γ-lactone moiety, indicating that this chemotype might serve as a basis for the further development of improved MptpB inhibitors.

In order to test the antimycobacterial potential of these compounds against human macrophages cell lines, polymersomes embedding the most active derivatives were assembled, starting from poly(2-(methacryloyloxy)ethylphosphorylcholine)-poly(2-diisopropylaminoethylmethacrylate copolymers, to ensure an efficient cytoplasmatic delivery.[4,5]

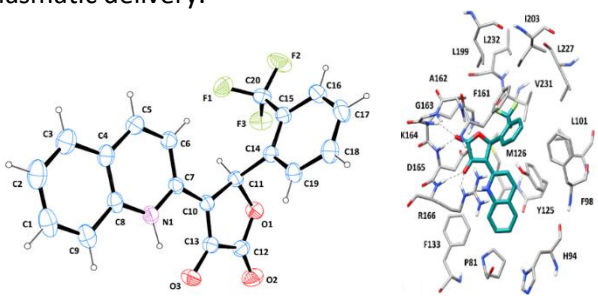


**Fig. 1**. ORTEP diagram of **1** (left) and its minimized average structure in complex with MptpB (right).

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PO-o62

# STRUCTURE-BASED OPTIMIZATION OF DUAL AChE-MAO B INHIBITORS: SCOUTING PHENYL RING BIOISOSTERES

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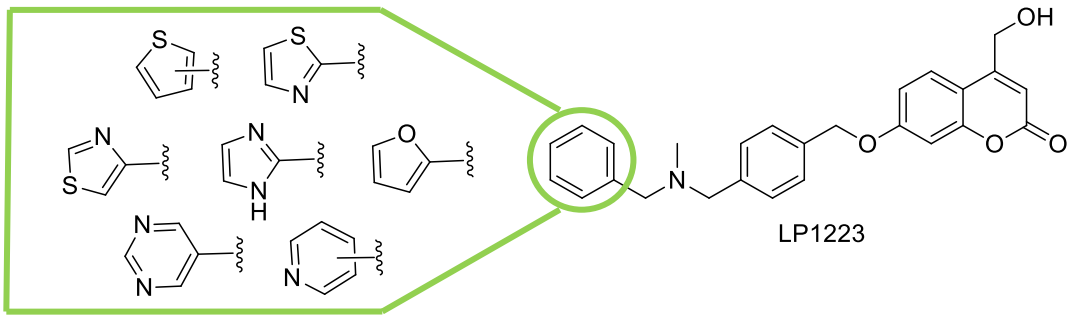
Alzheimer's disease (AD) is an age-related disease representing the most common form of senile dementia and triggering deficits of memory and other cognitive domains. The incidence of AD pathology doubles every 5 years after 65 years of age[1]. Due to multifactorial etiology, a more promising therapeutic perspective aims at acting simultaneously on two or more AD-related targets. According to this multitargeting concept, a large number of dual AChE-MAO B inhibitors have been previously investigated by some of us[2]. Among these compounds, LP1223 (Figure 1) was identified as a potent in vitro dual inhibitor showing poor drug-like character (i.e., low aqueous solubility at physiological pH, inadequate log$D_{7.4}$ for central drugs, and high lipophilicity)[3]. Therefore, current studies are focusing on improving hit drug-likeness while maintaining multitarget inhibitory potencies. Aided by the availability of X-ray crystal structures of human MAO B and mouse AChE in complex with LP1223[4], a bioisosteric approach addressed the replacement of the terminal phenyl ring, as a key structural template for drug-like and pharmacodynamic features. In particular the terminal phenyl ring has been replaced with five- or six-membered heteroaromatic rings in order to keep π–π stacking interactions with Trp-side chain in both targets (Trp119 in MAOB and Trp86 in AChE) and to improve aqueous solubility. Interestingly, a linear correlation between dual inhibition and the p$K_a$ of basic nitrogen was observed in SAR analysis.




| | $IC_{50}$ hAChE | $IC_{50}$ hMAOB | $Sol_{7.4}$ | log$D_{7.4}$ | CHI |
|---|---|---|---|---|---|
| LP1223 | 120nM | 10 nM | 13 µM | 3.81 | 99.3 |

**Figure 1.** Chemical structure of the reference compound LP1223 and preliminary drug-like data.

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PO-063

# DESING AND SYNTHESIS OF NEW HYBRID COMPOUNDS TARGETING BACTERIAL CARBONIC ANHYDRASES BASING ON A MULTITARGETING APPROACH

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Antibiotics have been proven effective in treating infections for hundreds of years. However, overuse of antibiotics has resulted in antimicrobial resistance (AMR) defined as the ability of microorganisms to survive and be viable under the influence of antimicrobial agents.[1] AMR was highlighted in a 2021 report from the WHO Global Antimicrobial Surveillance System. It is estimated that a total of 700,000 people die each year because of bacterial infection and resistance, and the burden of death would soar to 10 million by 2050 due to antimicrobial resistance unless action is taken.[2] Therefore, there is the need to develop new effective drugs acting against novel targets.

Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes which catalyze the hydration of carbon dioxide to bicarbonate and protons. As a consequence, and due to the high availability of $CO_2$ from metabolic processes, this reaction constitutes the basis of pH regulation in most living organisms.[3] Many pathogenic bacteria encode such enzymes belonging to the α-, β-, and/or γ-CA families in which they play crucial functions. As bacteria predominantly encode for β-class CAs, which are not present in vertebrates, these enzymes started to be considered as possible drug targets for obtaining antibacterials devoid of the resistance problems mentioned above, which affect most classes of antibiotics in clinical use. In the last decades, the multiple targeting approach was considered. This approach contemplates dual/multiple (hybrid) drugs, that is, compounds that incorporate in the same molecular entity at least two different chemotypes which hit diverse drug targets.[4] The multitargeting approach of CA inhibitors (CAIs) have been recently proposed. In detail, the CAIs moieties like sulphonamide have been hybridised with a variety of compounds like non-steroidal anti-inflammatory drugs, β-adrenergic blockers, and anticancer agents among others.[5]

Thus, basing on the multitargeting approach, we designed and synthesized a series of heterocyclic derivatives as CAIs endowed with antibacterial activities. In particular, the newly obtained compounds incorporate two diverse chemotypes, a sulphonamide moiety known for its CA inhibiting properties, and one based on the quinolinone scaffold. The data coming from the biochemical assays will be shown and discussed.

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PO-064

# NOVEL RIVASTIGMINE STRUCTURE-BASED HYBRIDS FOR THE POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an aging-relating neurodegenerative disorder. The origin of AD is still unknown, however several hypotheses have been formulated, including the cholinergic deficit, the aggregation of beta amyloid (Aβ) protein, oxidative stress, metal dyshomeostasis and recently the involvement of cannabinoid system.[1]

Currently, four symptomatic drugs have been approved by European Medicinal Agency (EMA) for the treatment of this form of dementia: three cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and a *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine.[2] Due to the multifactorial nature and complexity of AD, the design of multi-target agents could be a winning strategy for AD prevention and therapy.[2]

A series of hybrids presenting a portion miming donepezil condensed with aryloxyacetic acids, were reported in a recent published work.[3] All compounds were excellent inhibitors of cholinesterases *in vitro*, and besides, most of them were good inhibitors of Fatty Acid Amide Hydrolase (FAAH), the main catalytic enzyme of endocannabinoids. Results highlighted the multi-target properties of some compounds of the series.[3]

Using the same multi-target approach, in this work, a new series of hybrids were designed. These compounds contain the most promising aryloxy moieties[3] condensed with a rivastigmine-like fragment, differing for the substituents on terminal nitrogen atom and/or the position of carbamate on phenyl group. Rivastigmine was chosen because it is able to inhibit both cholinesterases, considering that recent studies have shown that the simultaneous inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) can be useful to control the progression of neurodegenerative diseases, including AD.[4] Figure 1 reports the general structure of these molecules.

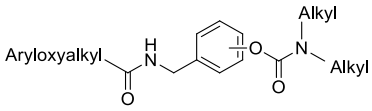


**Figure 1**

A representative part of the designed series was synthesized and tested *in vitro* as inhibitor of cholinesterases, FAAH and Aβ aggregation. Preliminary results will be presented in this communication, as well as a tentative of *in silico* rationalization.

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PO-065

# MODULATORS OF HUMAN MITOCHONDRIAL PROTEASE ClpP ACTIVITY FOR PEDIATRIC DIFFUSE PONTINE GLIOMA TREATMENT

Miciaccia, M.;[a] Ferorelli S.;[a] Scarcia, P.;[b] Carrieri, A.;[a] Rizzo, F.;[b] Loguercio Polosa, P.;[b] Perrone, M.G.[a] and Scilimati, A.[a]

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Diffuse intrinsic pontine glioma (DIPG) is an aggressive pediatric brainstem cancer and a leading cause of pediatric brain tumor-related death with the median survival of 10 months post-diagnosis. The sensitive location and infiltrative nature of the tumor makes full surgical resection unfeasible therefore targeted radiation has been the standard therapy to alleviate pain and transiently stabilize neurological functions.[1] DIPG resistance to commonly chemotherapeutic agents, provided a pressing need for the development of novel therapeutics for this malignancy. Meantime, the compound ONC201 was discovered to reduce DIPG tumors size in DIPG H3K27M mutated and now is in phase III clinical trials.[2]

Efforts to better understand DIPG related mechanism have proposed a new cellular target, being the mitochondrial caseinolytic protease P (ClpP). ClpP is found in both mammalian and bacterial cells. It is a highly conserved serine protease which combines with an ATPase to form a proteolytic complex responsible for the degradation of misfolded proteins at cellular level. In view of the potential therapeutic applications of ONC201 and other to-be-developed molecules, it is crucial to understand the molecular mechanisms linking ClpP activity with DIPG tumor onset and progression. Currently, the effects of the newly identified small-molecule modulators are under evaluation on ClpP enzymatic activity by using purified, recombinant human ClpP protease in a fluorogenic in vitro assay.[2] This study will help to gain insights into mitochondrial functions regulated by ClpP.

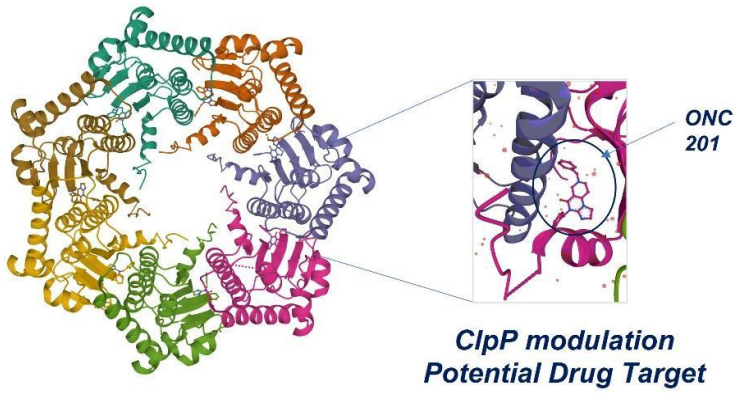


**Figure 1.** Human mitochondrial ClpP in complex with ONC201 (PDB ID: 6DL7).[3]

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PO-o66

# YEAST DISPLAY PLATFORM FOR THE DISCOVERY AND EVOLUTION OF GENETICALLY ENCODED CYCLIC PEPTIDES FOR THERAPEUTIC APPLICATIONS

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Cyclic peptides are a class of innovative drug molecules that represent a suitable alternative to small molecule and recombinant protein-based treatments. Cyclic peptides stand out for their unique proprieties including high selectivity and target-affinity, low toxicity and antigenicity, compatibility with chemical synthesis and biological high throughput screening [1-3]. Here, a yeast display platform for the generation and screening of large libraries comprising billions of different cyclic peptides was developed by combining random mutagenizes, fluorescence activated cell-sorting and NGS analyses [4]. The platform was applied to evolve peptide binders with desired affinity and selectivity towards different classes of protein targets. The described platform offers the possibility to rapidly identify and characterise peptide binders towards a broad range of pharmaceutical relevant targets (**figure 1**).

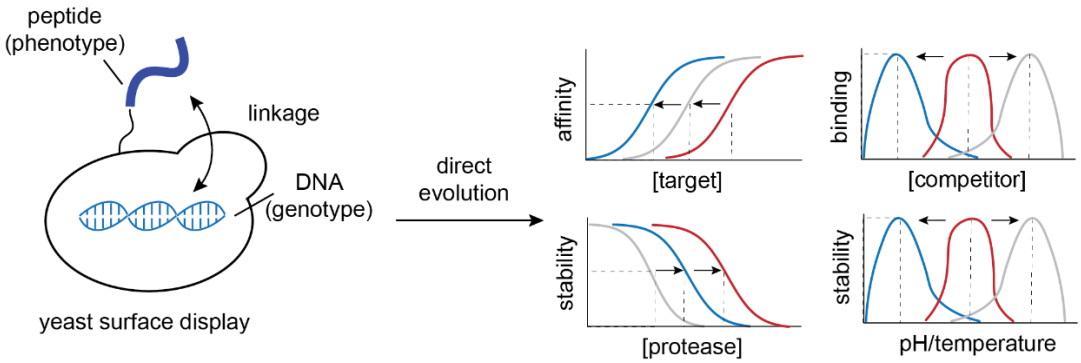


**Figure 1,** Schematic representation of yeast display technology that relies on a physical linkage between the display peptide sequence (phenotype) and its encoding DNA sequence (genotype). The yeast display platform via a direct evolution approach allows the selection and the engineering of key peptide proprieties, such as affinity, binding specificity, thermal and chemical stability.

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PO-067

# NOVEL BIOACTIVE AZITHROMYCIN-THIOSEMICARBAZONE CONJUGATES: DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY

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Macrolide antibiotics have been widely used for the infections causing by Gram positive and some Gram negative bacteria. Azithromycin is a semisynthetic macrolide antibiotic which possess excellent pharmacokinetic properties and good biological activity.[1] However, due to the emergence of multi-drug resistant bacteria there is a need to discover new, more effective antibiotics. In this work, new azithromycin derivatives where designed and synthesized.[2,3,4] Previous research[5,6] have shown that thiosemicarbazones have biological activity, hence they were used to modify azithromycin on three different positions (**Figure 1.**). These novel azithromycin-thiosemicarbazone conjugates are called the macrozones. Macrozones showed excellent *in vitro* activity against some susceptible and resistant bacterial strains, such as *S. pneumonia*, *S. pyogenes* and *S. aureus*. Here we present and discuss design, synthetic routes, characterization and biological activity of macrozones.

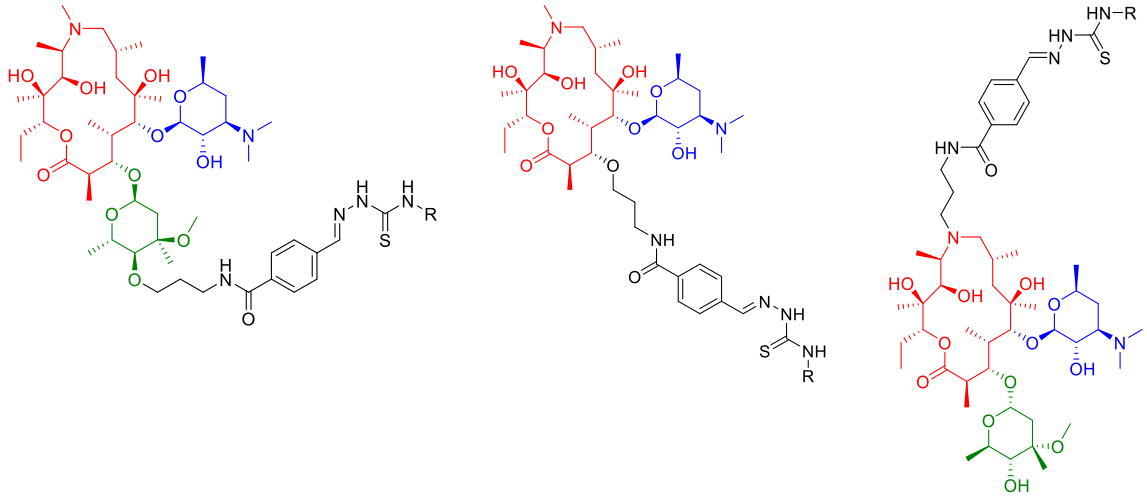


**Figure 1.** Structures of (a) 4''-macrozone, b) 3-macrozone and c) 9a-macrozone.

## Acknowledgments

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PO-o68

# DESIGN AND SYNTHESIS OF NEW DUAL INHIBITORS OF GSK-3β AND FYN TO COMBAT NEUROINFLAMMATION

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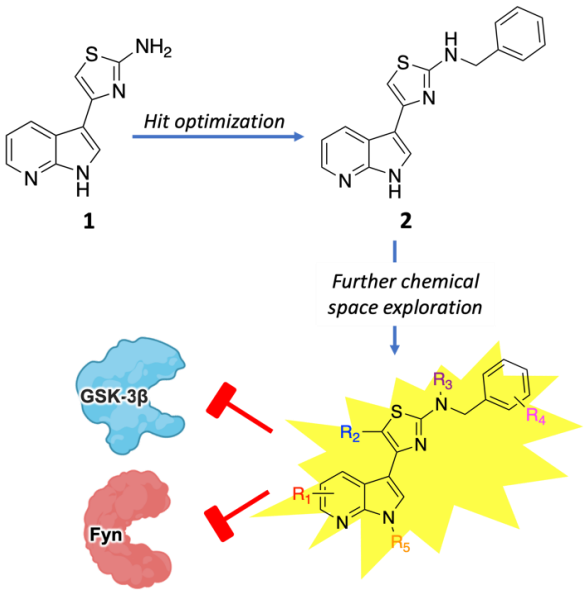
Neuroinflammation is a chronic pathological hallmark of Alzheimer's disease caused by the deposition of β-amyloid peptide (Aβ) and the hyperphosphorylation of tau protein (τ), which ultimately lead to the onset of neurodegenerative processes.[1] High levels of Aβ are responsible for the activation of astrocytes and microglia[2] and for the upregulation of many protein kinases, among which GSK-3β and Fyn play a relevant role. Indeed, these two kinases activate a cascade of events culminating in the transcription of pro-inflammatory genes and in the τ hyperphosphorylation, which is no longer able to ensure the axonal transport, thus leading to neuronal death.[1,3]




Since many biochemical pathways contribute to the development of neuroinflammation, the design of multitarget-directed ligands, *i.e.* single molecules capable of modulating two or more biological targets simultaneously, may represent a valuable pharmacological approach.[4]

In this view, with the aim to discover new dual GSK-3β and Fyn inhibitors we first identified a hit compound **1** (figure 1) through a virtual screening campaign. Its structure is based on a 7-azaindole-3-aminothiazole scaffold and presented a weak micromolar inhibition in *in vitro* enzymatic assays on both kinases, although unbalanced towards GSK-3β. Therefore, we undertook a hit optimization campaign from which compound **2** (figure 1), bearing a benzyl moiety on the amino group of thiazole, emerged as the compound with the most balanced inhibitory profile between the two kinases. Further modification on derivative **2** allowed us to increase the potency into the low micromolar and to improve the activity against Fyn kinase. Moreover, the most promising compounds, tested on rat primary cultures of cerebellar granule neurons, do not display cytotoxicity up to 25 μM and besides they already exert a neuroprotective action after a degenerative stimulus at 5 μM concentration, which is consistent with the $IC_{50}$ values obtained from the enzymatic assays.

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PO-069

# DESIGN AND SYNTHESIS OF NEW HAPLOID GERM CELL-SPECIFIC NUCLEAR PROTEIN KINASE (HASPIN) INHIBITORS

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Histone modifications are important events in tumoral cells and these biological processes are proving potential targets for the development of new anticancer agents.
Histone H3 associated protein kinase (Haspin) is a 798 amino acid serine/threonine protein kinase with a critical function in mitosis. The enzyme phosphorylates threonine-3 on histone H3 during mitosis, enabling proper cohesion of chromatin and metaphase alignment, and in this way ensuring the normal continuation of the cell cycle. [1,2] Since Haspin is highly expressed in proliferating cells,[3] we synthetized a series of molecules with indolic scaffold as potential Haspin inhibitors for a new epigenetic anti-cancer treatment.
The design was based on the results obtained from the evaluation of the cytotoxic activity of a series of tri-substituted indole derivatives on different cancer cell lines as multi-target kinase inhibitors. [4] The compound INDO11M, which showed remarkable inhibitory activity against Haspin, was used as hit compound for a computer-aided design of new molecules potentially active against mitotic kinase.
In a preliminary evaluation, carried out by a FRET-based assay for analyzing their inhibitor activity against Haspin, several of the synthesized compounds showed interesting $IC_{50}$ values in the sub micromolar and nanomolar range. These results encourage our group to plan *in vitro* supplementary studies for evaluating their cytotoxic activity in a cell environment.

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*PO-070*

# OXIDATIVE STRESS MEASUREMENT FOR EARLY DIAGNOSIS OF METABOLIC AND CNS DISEASES

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Oxidative stress induces an imbalance between the concentration of Reactive Oxygen Species (ROS) and the activity of antioxidant defence mechanisms, in favour of the first one. High oxidative stress plays a fundamental role in various diseases such as cancer, neurodegenerative, cardiovascular, inflammatory diseases, and intoxications. This condition could depend by factors such as aging, intense physical activity, junk food and unhealthy lifestyles. The peroxidation of arachidonic acid, as a component of cell membranes, is an exemplary model of cell injury. Malondialdehyde (MDA) is formed due to peroxidation of fatty acid, and it is involved in further cascade reactions with proteins and DNA. However, the physiological levels of ROS are important for maintaining correct cellular and molecular functions. The mechanisms through which antioxidants interfere with ROS, such as during the fatty acid peroxidation, they will support us to provide a rational approach that would bring benefits in the pharmacological field. The aim of this presentation is to clarify the points of the biochemical pathways, where new antioxidant activity molecules could reduce oxidative stress. In the present study will be present oxidative stress evaluations that will be carried out in four groups of subjects classified as following reported: healthy (control group), smokers, subject with neurodegenerative (CNS) and cardiovascular diseases.

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PO-071

# BINDING AFFINITY DETERMINATION OF PACLITAXEL-EVANS BLUE DERIVATIVES TO HUMAN SERUM ALBUMIN BY SURFACE PLASMON RESONANCE

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For decades, the systemic treatment of triple negative breast cancer (TNBC) has relied exclusively on chemotherapy with limited effectiveness. To address this unmet clinical need, intense research has been focusing on reshaping the treatment protocol for this disease. Growing evidence suggests that combination therapy represents a central pillar for tumour treatment. This strategy enables the targeting of key pathways in a characteristically synergistic or an additive manner and provides a viable strategy to overcome multidrug resistance. However, combination therapy deals with the same drawbacks as mono therapy: poor bioavailability, non-selective cytotoxicity, and difficulties in achieving therapeutic drug concentrations.[1] Therefore, extensive efforts aim at developing drug co-delivery systems that rely on macromolecules or nanoparticles, which are able to release anti-cancer drugs primarily at tumor sites, minimizing toxicity in healthy tissues, exploiting the so-called enhanced permeability and retention (EPR) effect.[2] EPR consists of the accumulation of high-molecular weight compounds in tissues exhibiting increased vascular permeability, as cancer.[3] One of the most promising macromolecules that has been employed as carrier is human serum albumin (HSA), due to its inherent safety, bioavailability, and long half-life. [4] In this study, it has been evaluated whether two designed paclitaxel (PTX) derivatives, named A and B, were able to interact with HSA. Both compounds bear PTX prodrugs, a cytotoxic chemotherapeutic agent that, according to recent literature, can re-establish immunosurveillance by triggering immunogenic cell death (ICD).[5] These compounds will constitute an innovative and biomimetic nanobinder. Compound A and B were specifically designed to possess an Evans blue (EB) fragment, an azo dye and a clinical diagnostic agent with high affinity to HSA ($K_D$ = 2.5 µM).[6] In the present work, Surface Plasmon Resonance (SPR) technology was performed to determine binding affinity of compounds A and B to HSA with reference to the modified Evans blue fragment. A sensor chip bearing a carboxy-methyldestrane layer on the gold surface was prepared by using a previously developed and validated procedure. In brief, in both sample and reference flow cells, an anti-HSA antibody was immobilized by amine coupling procedure. Functionalization of both flow cells enabled to zero out non-specific interaction of the analytes with the surface antibody. The sensor chip was, then, used for immunocapturing HSA. Due to the expected low dissociation rate of the compound-HSA complex, a single-cycle kinetics was used for the binding affinity determination. In this protocol sequential injections of increasing concentrations of the analyte were injected over the ligand, without performing any dissociation or regeneration between each sample concentration. This allows for the characterization of binding to ligand surfaces that are difficult to regenerate. Interaction between the synthon EB derivative and HSA gave a $K_D$ value of 1.29±0.24 µM, which is comparable with that reported in the literature for EB. SPR analysis performed to determine binding affinity of compounds A and B showed that both designed molecules are able to strongly interact with HSA and are, hence, suitable for being included in tailored delivery systems.

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PO-072

# IDENTIFICATION OF THE MOLECULAR REQUIREMENTS OF TAU AGGREGATION INHIBITORS THROUGH CHEMOINFORMATICS ANALYSES

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Tau is a highly soluble, cytoplasmatic protein that plays a central role in the regulation of the dynamic equilibrium of microtubules, especially in the neuronal cells. Recent evidences have demonstrated that aberrant metabolism or hyperphosphorylation of this protein is involved in the physiopathology of around 26 neurodegenerative disorders (*i.e.*, tauopathies), Alzheimer's disease (AD) being one of the most common.[1,2] Increased interest has arisen around the identification of therapeutics targeting tau,[3] especially considering that tauopathies mainly affect elderly people. At present, there are neither available therapeutic remedies able to reduce Tau aggregation, nor are there any structural clues or guidelines for the rational identification of compounds preventing the accumulation of protein aggregates. With the aim to identify a set of structural features and molecular properties characterizing compounds endowed with tau anti-aggregation activity, we performed extensive chemoinformatics analyses on ligands reported in ChEMBL (Figure 1). In particular, we performed MACCS, ECFP4, AtomPairs and TopologicalTorsion fingerprints-based similarity estimations identifying a high structural variability on reported Tau ChEMBL ligands. Besides, comparisons of a set of 118 molecular descriptors of active (Potency ≤500nM) and inactive (Potency inactivity thresholds ≥1μM, ≥5μM, ≥10μM and ≥20μM) compounds was also performed, allowing the identification of structural properties that are mainly present among ligands exhibiting anti-aggregation activity towards this target. Fragments composition analyses were also performed on Tau ligands, facilitating the identification of combinations of chemical moieties frequently present in Tau anti-aggregation ligands. Interestingly, many of these fragments were arranged in recurring frameworks, some of which were clearly present in compounds currently under clinical investigation. This work represents the first in-depth chemoinformatics study of the molecular properties, constituting fragments and similarity profiles, of known Tau aggregation inhibitors.[4] The results might be useful to researchers interested in both *de novo* design of anti-aggregation ligands and drug repurposing against Tau.[4]

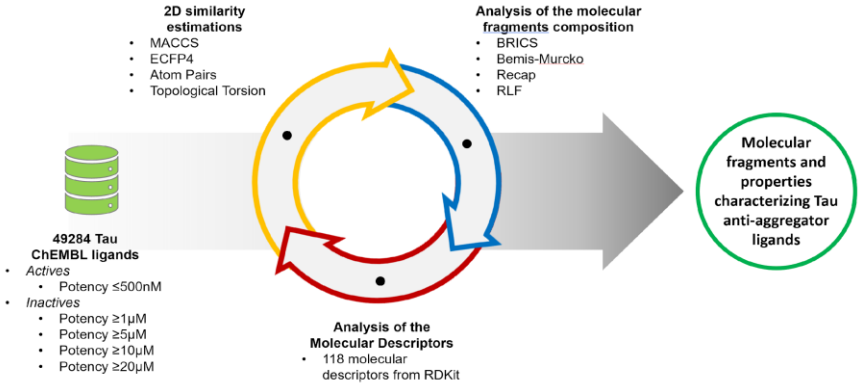


**Figure 1.** Chemoinformatics analyses on Tau ligands reported in ChEMBL.

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PO-073

# AMG 837: A PROMISING HIT AS AN ANTIBACTERIAL AGENT AGAINST STAPHYLOCOCCUS AUREUS

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New classes of antibiotics are urgently needed to tackle multidrug-resistant bacteria, such as *Staphylococcus aureus*.[1,2] In this regard, we screened selected set of PPARα/γ, GPR40, and GPR120 modulators against *S. aureus*. AMG 837 (**1**),[3] a potent, orally bioavailable GPR40 agonist, was identified as a possible hit with good inhibitory activity against free-floating planktonic cells and within biofilms of *S. aureus*. However, to avoid possible liver toxicity issue associated with AMG 837 due to its high lipophilicity (CLog*P* = 6.82),[4,5] we synthesized five structurally similar analogs having lower lipophilicity (CLog*P* = 3.52-5.82) by replacing central phenyl ring of AMG 837 with selected five-membered heterocyclic rings. However, when tested these analogs were found to be inactive. Although disappointed with this outcome, in due course, we aim to screen the above-mentioned five analogs against other multidrug-resistant bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa.* Moreover, we plan to synthesize additional analogs by modifying carboxylic acid side chain of AMG 837.

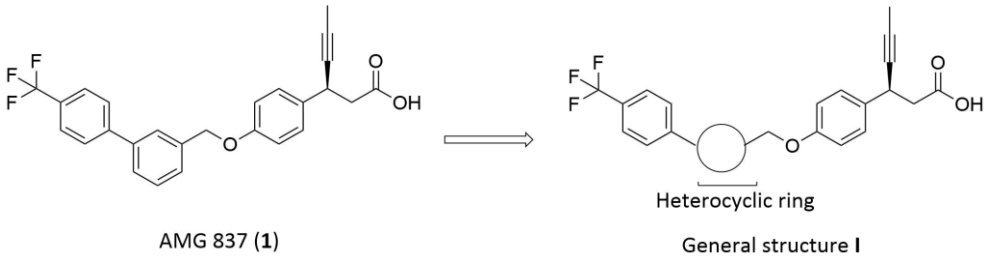


**Fig. 1.** Structure of AMG 837 (**1**) and its related analogs as general structure **I**.

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PO-074

# MICROSAMPLING FOR BIOMARKER INVESTIGATION IN ANIMAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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From a drug discovery perspective, biomarkers are exploited to identify novel drug targets aiding preclinical drug development. Biomarkers can also provide important links between preclinical disease models and human patient population, highlighting potential therapeutic targets. Within bioanalysis, technologies for the miniaturised sampling of biological matrices bring inherent advantages that can expand and accelerate different types of studies, with benefits for researchers, clinicians and patients.[1] Amyotrophic lateral sclerosis (ALS) is a neurodegenerative neuromuscular disease for which there is currently no cure and no effective treatment to halt or reverse the disease progression. The aetiology is largely unknown, but several pathogenetic mechanisms have been proposed, including CNS neuroinflammation driven by unbalanced immunoregulatory responses. Among the main physiological immunoregulatory mechanisms, the catabolism of the aminoacid tryptophan (TRP) has a crucial role and it has been shown how several TRP metabolites are involved in many neurological disorders.[2]
Based on these premises, the purpose of this study within a multidisciplinary research project is aimed at designing and developing ad-hoc analytical approaches based on microsampling and miniaturised pretreatment strategies. These were optimised in order to be exploited for the quali-quantitative assessment of several TRP-related compounds in advanced biological samples, including kynurenine and methoxyindole pathway metabolites and gut microbiota TRP metabolites. From the bioanalytical point of view, the availability of limited sample volumes and the perspective of reducing solvents and reagents in the framework of sustainable protocols, make advanced microsampling technologies particularly attractive and promising, also in the framework of the 3Rs principle (Replacement, Reduction and Refinement) for animal research. An original miniaturised sample collection workflow has been developed based on whole blood volumetric absorptive microsampling (VAMS). The developed and finely tuned protocol has been coupled to LC-MS/MS for quantitative evaluations, while high resolution mass spectrometry (HRMS) was exploited to further confirm target analyte identity. Analyte separation was achieved by exploiting an optimised chromatographic RP system, while ESI-MS source ionisation was set-up and optimised both in positive and negative mode. This approach has been fully validated obtaining satisfactory results, thus the developed analytical platform represents a promising and versatile tool allowing the quali-quantitative evaluation of a broad panel of TRP-related compounds in microsamples in dried form. Within this project, the methodology is being applied for the analysis of miniaturised samples coming from mice bearing ALS and wild-type controls and will allow to evaluate the presence of specific TRP metabolites that could potentially have a role in the onset and progression of the disease and hopefully address new promising therapeutic targets for ALS.
*This research was financially supported by the Research Projects of National Relevance (PRIN) 2017 funds (Italian Ministry of Education, University and Research), Project 20173EAZ2Z.*

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PO-075

# DISCOVERY OF PYRROLO-PYRIMIDINE AND PURINE-BASED HDAC6 INHIBITORS BEARING DIFFERENT ZINC-BINDING GROUPS FOR THE TREATMENT OF AGGRESSIVE PROSTATE CANCER

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Prostate cancer (PC) is among the most frequent types of tumors and one of the main causes of mortality in men.[1] Finding effective treatments for patients with aggressive prostate cancer (PCA) remains a challenge, mainly due to the insurgence of drug resistance.[2] Interestingly, recent studies demonstrated that the activity of Histone Deacetylase 6 (HDAC6) is linked to androgen receptor hypersensitivity and nuclear localization in prostate cancer. Moreover, HDAC6 is overexpressed in several types of cancer and facilitates cell migration and proliferation. Therefore, HDAC6 emerged as an important anticancer drug target. Following our interest in developing HDAC6 inhibitors,[3-7] we have designed, synthesized and tested twenty-nine novel HDAC6 inhibitors based on three different zinc binding groups (*i.e.*, trifluoromethyloxadiazole – TFMO, hydroxamic acid – HA, 2-mercaptoacetamide – MCA). These warheads were conveniently tethered to variously substituted phenyl linkers and decorated with differently substituted pyrrolo-pyrimidine and purine cap groups (Figure 1). Remarkably, several of the designed molecules showed nanomolar to sub-nanomolar inhibitory activity and selectivity against HDAC6. Docking and molecular dynamics were performed to help rationalizing the structure-activity relationships of the compounds. Molecules bearing the TFMO and HA zinc binding groups showed promising anti-proliferative activities, specific HDAC6 targeting in PCA cells, and tumor selectivity. Representative compounds of the two series were tested for solubility, Caco-2 cell permeability and metabolic stability, demonstrating favorable *in vitro* drug-like properties. Migration assays performed on the most interesting compounds revealed that some of them inhibited the invasive behavior of PCA and castration-resistant prostate cancer cells. Altogether, this study led to the identification of several potent HDAC6 inhibitors, some of which proved to be very promising also in terms of anti-proliferative and anti-migration properties. One compound combined potent HDAC6 inhibitory activity and selectivity, favorable drug-like properties, excellent anti-proliferative activity and marked anti-migration properties on PCA cells, thus representing a valuable preclinical candidate.

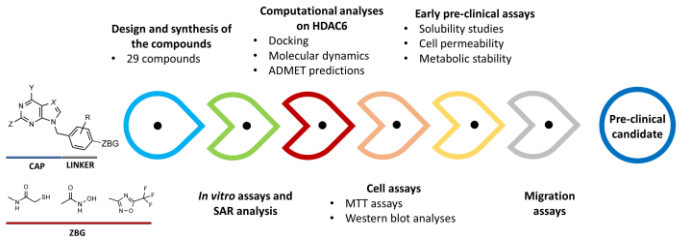


**Figure 1.** Workflow adopted for the development of the compounds.

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PO-076

# INVESTIGATING HYDROXYTYROSOL DERIVATIVES AS MULTIMODAL AGENTS FOR TREATING NEURODEGENERATIVE DISEASES

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Alzheimer's disease (AD) is the most common neurodegeneration affecting elderly people worldwide. The AD hallmarks are deposition of extracellular β-amyloid (Aβ) peptide into plaques, low levels of acetylcholine (ACh), oxidative stress and bio-metal dyshomeostasis. The increase of butyrylcholinesterase (BChE) during the disease progression highlighted BChE inhibition as possible therapeutic strategy, whereas the inhibition of monoamine oxidases (especially MAO B) could increase the level of neurotransmitters and mitigate neuronal oxidative damage.

Our research group has long investigated the chemical space around the indolinone core, disclosing aryl hydrazone of isatine as potent *in vitro* inhibitors of Aβ aggregation, acting at different stages of the fibrillogenesis.[1] Recently, we reported azepino[4,3-*b*]indole-based selective inhibitors of BChE endowed with additional neuroprotective activities,[2] and optimized tetrahydrochromeno[3,2-*c*]pyridinone derivatives[3] having potential as antioxidant, anti-inflammatory, anti Aβ-fibrillization and metal-chelating agents, MAO B inhibitors and free radical scavengers.

Among the natural compounds, Hydroxytyrosol (HTyr), a major polyphenolic component of olive oil, is endowed by multiple pharmacological activities. HTyr has been studied for its protective role in neurodegenerative diseases, due to its capacity of reducing the oxidative stress at neuronal level and protecting neurons against amyloid-β induced toxicity.

In our ongoing research program (Figure 1), we designed, synthesized, and tested *in vitro* a number of HTyr-based hybrid molecules and double prodrugs with isatine hydrazones, azepino-indole and chromeno-pyridinone. ChE/MAO inhibition and antioxidant properties were determined *in vitro*, and structure-activity relationships studied for supporting new design of MTDLs with improved biological properties toward AD-related drug targets.

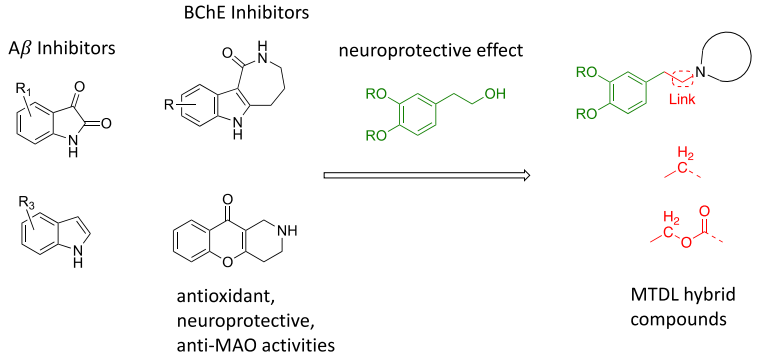


**Figure 1.** Design strategy of new hybrid compounds and double prodrugs of HTyr joint or linked with isatine, indole, tetrahydro-azepino-indole and tetrahydro-chromeno-pyrimidinone.

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PO-077

# NEW AKR1C3 INHIBITORS WITH BENZOISOXAZOLE CORE: DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY TO TARGET PROSTATE CANCER

Pippione, A.C.;[a] Iakovleva, M.;[a] Vottero, C.;[a] Sainas, S;[a] Kilic-Kurt, Z.;[b] Rolando, B;[a] Kovachka, S; Spyrakis, F; Buschini, A.;[c] Montalbano, S.;[c] Oliaro Bosso, S.; Lolli, M.L[a] and Boschi, D.[a]

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Since 2017 our group has developed several molecules able to inhibit the steroidogenic enzyme aldo-keto reductase 1C3 (AKR1C3), that is considered an attractive target in Castration Resistant Prostate Cancer (CRPC): AKR1C3 catalyses some key steps of biosynthesis of androgens testosterone and DHT and, at the same time, it is implicated in resistance to several anticancer drugs.[1]
We recently report three series of AKR1C3 inhibitors containing hydrolated azoles, derived from modulation of the not selective inhibitor flufenamic acid.[2] Here, we describe the bioisosteric approach used to discover the *hit compounds* and how, combining crystallographic experiments and *in silico* guided design, we finally obtained very potent AKR1C3 inhibitors with notable activity against CRPC models.
We also investigated the effects of the best AKR1C3 inhibitors in combination with two drugs currently used for the clinical treatment of CRPC: abiraterone (CYP17A1 inhibitor) and enzalutamide (AR antagonist), for whose AKR1C3 activation is a critical mechanism of resistance. The obtained results of this combination study showed enhanced effects, suggesting the effectiveness of the combination therapy with these elective drugs to increase their efficacy.
*In silico* design, synthesis, enzymatic inhibition and biological evaluation against tumoral and non-tumoral cells of the new series of AKR1C3 inhibitors are here described and discussed.

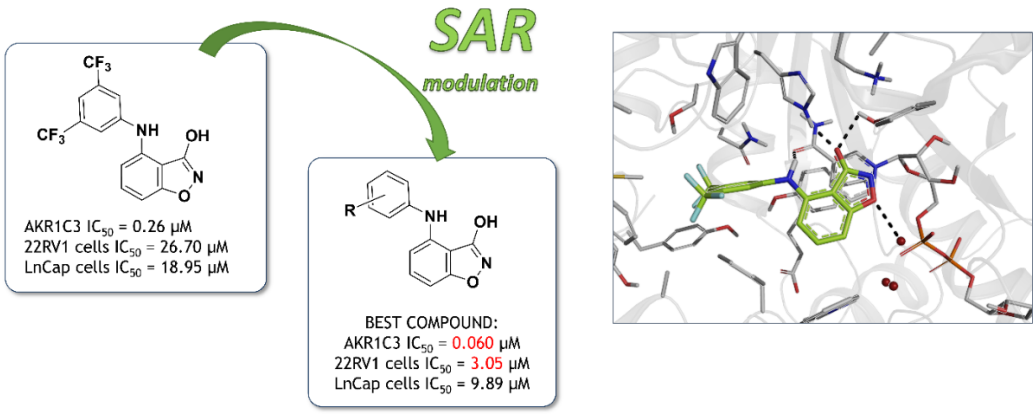


**Figure 1.** Design of new AKR1C3 inhibitors with benzoisoxazole core.

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PO-078

# LOOKING FOR NEW TOOLS TO COUNTERACT NEURODEGENERATION. DESIGN AND SYNTHESIS OF HETERODIMERIC LIGANDS TARGETING SIGMA1 RECEPTOR AND TSPO

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Neurodegenerative diseases are complex, multi-factorial and debilitating disorders that represent one of the main therapeutic challenges nowadays. From a medicinal chemistry standpoint, the identification and validation of novel therapeutic targets and the use of multi-target directed ligands (MTDLs) may lead to the discovery of new effective agents against neurodegenerative diseases.[1] Our work in this field is aimed at developing dual ligands for targeting the Sigma1 Receptor (S1R) and the 18kDa Translocator Protein (TSPO). S1R is a ligand-operated molecular chaperone localized at the mitochondria-associated endoplasmic reticulum membrane (MAM) and highly expressed in the central nervous system (CNS). S1R agonists are known to promote neuroprotection and neuroplasticity.[2] TSPO is a transmembrane protein localized in the outer mitochondrial membrane, where it takes part to several pathways affecting cells survival and is upregulated in microglia under inflammatory conditions. It is widely used as diagnostic target to highlight neuroinflammation via PET imaging, but its therapeutic potential is still unexplored.[3] Given these premises and considering the convergence of S1R and TSPO pathways on the modulation of mitochondrial function, targeting both receptors could lead to an enhanced therapeutic potential and long-lasting neurosupportive effects. To probe this hypothesis, we developed a series of dual S1R-TSPO ligands based on the general structure reported in Figure 1. Briefly, our in-house developed S1R agonist RC-33, and the clinically validated TSPO ligand PK-11195 were used as model compounds for designing two moieties selective for each target. These structures were tethered by different types of linkers. The design and synthesis were driven by our previous experience on bivalent S1R ligands, with an eye on green chemistry and sustainability.[4,5] The compound series is now under preliminary biological investigation.

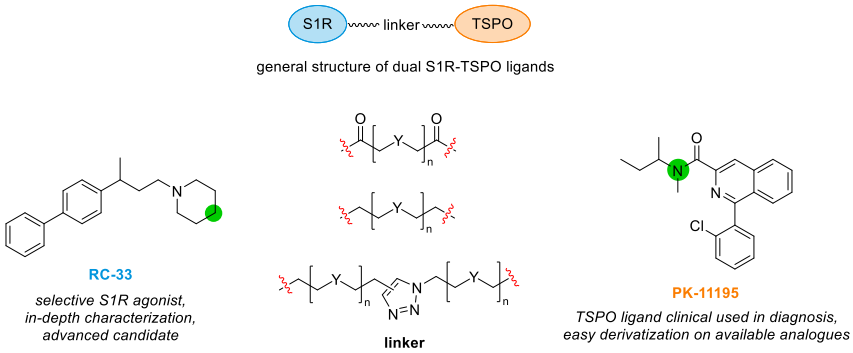


**Figure 1.** General structure of dual S1R-TSPO ligands and model compounds for the moieties interacting with the targets: RC-33 for the S1R-binding moiety and PK-11195 for the TSPO-binding moiety. Green circles represent the linker attachment points.

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PO-079

# SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL HEME OXYGENASE-1 MODULATORS AS POTENTIAL FERROPTOSIS INDUCERS

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Heme oxygenase (HO) is an intracellular enzymatic system responsible for heme degradation in stoichiometric amounts of ferrous iron, carbon monoxide, and biliverdin[1]. Among the two main isoforms identified so far, HO-1 is strongly induced under cellular stressful conditions and its expression is transcriptionally regulated by the Nrf2/Keap1 axis. HO-1, as the main target protein of Nrf2, has been demonstrated to be a useful target for the treatment of pathologies related to an unbalanced oxidative stress status. Moreover, HO-1 can exert either a cytoprotective or a detrimental action in different types of cancers[2,3], depending on the specific cellular conditions. Recent findings[4] suggest a prominent role of HO-1 induction in ferroptosis, a newly discovered form of cellular death triggered by iron accumulation and lipid peroxidation[5]. Herein we report the design, synthesis and preliminary biological evaluation on breast cancer cells of novel HO-1 inducers whose chemical structure derives from the natural compound caffeic acid phenethyl ester (CAPE). Preliminary biological studies showed CAPE's ability to increase HO-1 protein levels and its potential implication in ferroptotic cell death. Newly synthesized derivatives showed more potent antiproliferative effect compared to the parent compound and were able to induce both HO-1 protein expression and enzymatic activity. Ferroptosis mechanism was assessed on the most promising compounds by using Ferrostatin-1, a well-known ferroptotic inhibitor, and by measuring lipid peroxidation levels (LOOH). Obtained results suggest a potential implication of ferroptosis in the mechanism of action of the newly synthesized compounds. Further details will be shown at the meeting.

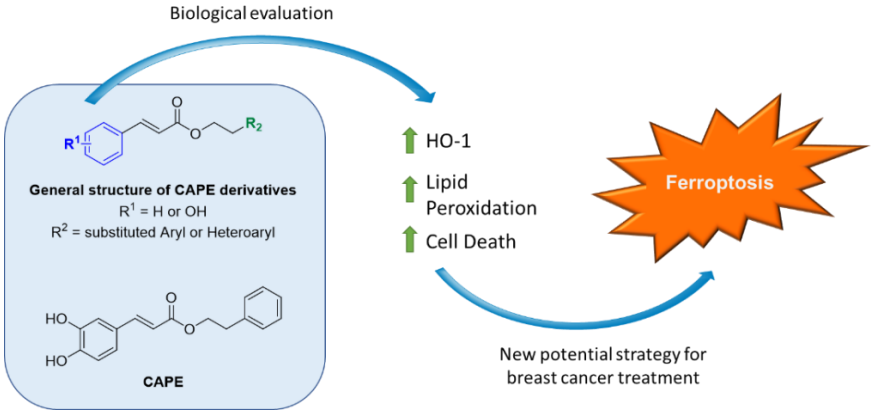


**Figure 1.** General structure of newly synthesized derivatives and biological evaluation.

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PO-o8o

# PROTAC-INDUCED GLYCOGEN SYNTHASE KINASE 3B DEGRADATION

Santini, A.;[1] Pruccoli, L.;[1] Guardigni, M.;[1] De Simone, A.;[2] Spyrakis, F.;[2] Frabetti, F.;[3] Andrisano, V.;[1] Bolognesi, M. L.;[4] Tarozzi, A.;[1] Milelli, A.[1]

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Glycogen synthase kinase 3β (GSK-3β) is a multifunctional serine/threonine protein kinase involved in several physiological and pathological conditions.[1] GSK-3β is found to be hyperactivated in the brain of Alzheimer's disease (AD) patients, and compelling evidence supports that it is the main tau kinase involved in AD's pathology.[2] Numerous studies have demonstrated the effectiveness of GSK-3β inhibitors in different models of neurodegenerative diseases.[3] However, developing specific and safe kinase inhibitors is particularly puzzling and, although kinase inhibitors have been widely applied in the clinic, growing evidence of off- and on-target toxic effects as well as increasing phenomenon of drug resistance stimulate the request of new generation of drugs.[4]

In the last years, a new ground-breaking approach to modulate the activity of a given protein of interest (POI) has appeared. In this approach, agents called PROteolysis TArgeting Chimeras (PROTACs) are used to control proteins level rather than modulate their function.[5] From a medicinal chemistry point of view, PROTACs are heterobifunctional molecules consisting of a ligand that binds the POI connected via a linker to a recruitment moiety for an E3 ubiquitin ligase.

In this study, a series of heterobifunctional small molecules PROTACs were designed and synthesized based on E3 ubiquitin ligase cereblon (CRBN) and using as POI-recruiter two different GSK-3β binding elements, deriving from the GSK-3β inhibitors SB-216763 and Tideglusib (Figure 1).[6] The biological activity of such GSK-3β-directed PROTACs will be discussed.

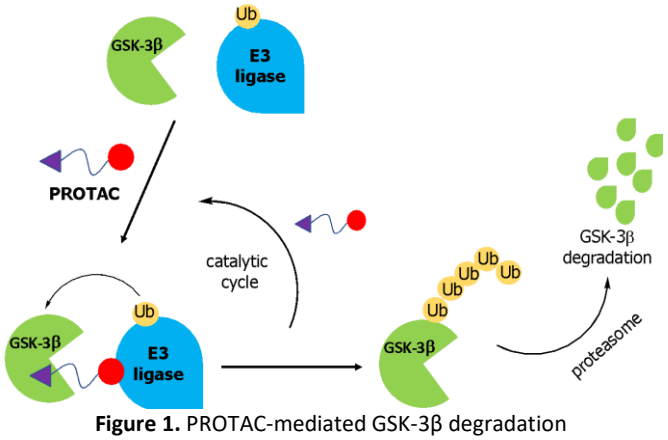


**Figure 1.** PROTAC-mediated GSK-3β degradation

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PO-081

# DEVELOPMENT OF SMALL PEPTIDE-BASED SARS-COV-2 $M^{PRO}$ INHIBITORS AS ANTIVIRAL AGENTS AGAINST COVID-19

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SARS-CoV-2 main protease ($M^{pro}$) is a cysteine protease involved in the processing of viral polyproteins to afford non-structural individual functional proteins. Due to the lack of homologous enzymes in human cells, $M^{pro}$ could be considered an ideal target for the development of anti-COVID-19 agents.[1] Among the small set of drugs authorised for the treatment of COVID-19, PF-07321332, also known as Nirmatrelvir (Paxlovid®), reversibly inhibits this viral protease.[2]

In this context, we recently carried out a virtual screening campaign against $M^{pro}$ using our in-house database of potential inhibitors and two hit compounds bearing a methyl vinyl ketone warhead have been identified.[3] Starting from these results, we focused our efforts on the development of small peptide-based Michael acceptors (Figure 1). In the new designed molecules, the methyl vinyl ketone warhead was kept unchanged, whereas, at the P1 site, a γ-lactam glutamine surrogate was introduced, considering its essential role for the binding affinity. At the P2 position, a small set of amino acids was rationally introduced, meanwhile, with regard to the P3 site, the new molecules carry differently substituted aromatic rings anchored by an amide or carbamate bond.

The novel Michael acceptors were fully characterized for their inhibitory properties: with a few exceptions, the new compounds showed $K_i$ values in the nanomolar range towards the target enzyme, and the most promising inhibitors exhibited single-digit micromolar $EC_{50}$ values against SARS-CoV-2 infected cells. Selectivity over a panel of appropriate viral and human proteases was assessed, as well as, the toxicity towards mammalian cells.

Moreover, to gain insight into the novel inhibitors' binding mode, molecular-docking experiments have been performed.

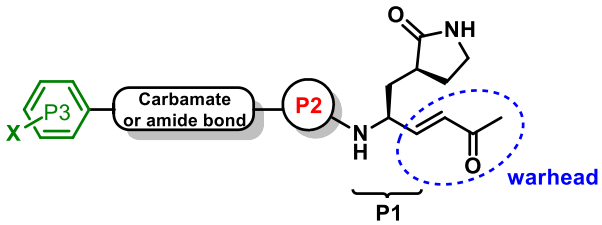


**Figure 1.** Chemical structure of the new Michael acceptors targeting SARS-CoV-2 $M^{pro}$.

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PO-o82

# NEW INSIGHTS INTO THE STRUCTURE–ACTIVITY RELATIONSHIP OF BENZOTHIAZEPINONE DERIVATIVES AS NCX3 MODULATORS

Scognamiglio, A.;[a] Corvino, A.;[a] Secondo, A.;[b] Pannaccione, A.;[b] Tedeschi, V.;[b] Piccialli, I.;[b] Santagada, V.;[a] Perissutti, E.[a] and Severino, B.[a]

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In the last 30 years, the occurrence of $[Na^+]_i$ and $[Ca^{2+}]_i$ dyshomeostasis has been reported in several neurodegenerative diseases either at neuronal or glial components. Altered expression and activity of NCX isoforms have been also demonstrated in stroke, multiple sclerosis (MS), amyotrophic lateral sclerosis, SMA, Parkinson's disease, and Alzheimer's disease.[1] Although the role of each isoform is still under examination, many evidence lines point toward a neuroprotective effect of NCX3 activation in most of these neurodegenerative diseases. In the light of this, we have designed several pharmacomodulation of the 1,4-benzodiazepinic nucleus which was highlighted as one of the scaffolds responsible for the enhancing or the blockade on the different NCX isoforms (Figure 1). In order to improve potency and selectivity for this target, we have synthesized, by standard and microwave assisted heating, and characterized novel 1,4-benzothiazepinonic derivatives of CGP37157, which is already described as a mitochondrial NCX (mNCX) blocker with a poor selectivity.[2] On the basis of SAR studies on NCX modulators, the benzothiazepinonic nucleus, variously substituted with EWG groups, has been linked in position 1 to a cyclic amine via an acetyl spacer.[3] Here to investigate the interaction and explore the possible effect of stereoisomery on the affinity towards the selected target, we used the 2-methylpiperidine as racemic mixture and the enantiomerically pure form: (R)-2-methylpiperidine and (S)-2-methylpiperidine. The compounds, named **CGP_1**, **2** and **4**, as racemic mixtures and (R)/(S) enantiomers, have been screened on NCX3 isoform activity by a high-throughput screening approach on BHK cells singly expressing this isoform. Identification of the newly synthesized compounds, enhancing NCX reverse mode or inhibiting NCX activity, was done by measuring their ability to increase, or decrease, the $Na^+$-free-dependent $Ca^{2+}$ level above the mean of basal value. Additionally, the compounds were functionally characterized by patch-clamp electrophysiology and Fura-2AM video imaging. In this way, we have identified nine pharmacological modulators of NCX3, able to change the calcium currents in reverse mode; among them, **CGP_1(S)** has shown the greatest potency to increase the NCX3 activity. All compounds linked with the (S)-2-methylpiperidine strongly enhanced the reverse and forward modes of operation of NCX3, instead the enantiomers with (R)-2-methylpiperidine showed less activity. Moreover, **CGP_4(R)** has demonstrated a strikingly inhibitor activity, in contrast to its (S) enantiomer, confirming the influence of the stereochemistry on the biological activity. Therefore, futures studies will investigate the influence of the stereogenic C-5 center on the pharmacological activity.

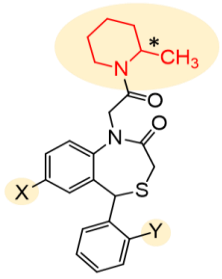


**Figure 1.** General structure of the synthesized compounds

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PO-083

# RS6077 AS NOVEL AND SELECTIVE GROWTH INHIBITOR OF HUMAN CANCER CELL LINES AND LYMPHOMA TUMOR

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In this project, we first selected (1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrrol-3-yl)(3,4,5-trimethoxy-phenyl)methanone (**1**, compound RS6077) as a new pyrrole tubulin polymerization inhibitor (chart 1). In previous work, we noted that the presence of a 3-amino group of the 1-phenyl ring of 3-aroyl-1-arylpyrrole (**2**) increases the inhibition of MDR cell lines and suppresses the Hedgehog signaling pathway.[1] Highlighted the effect of nitrogen atom we report the synthesis and first disclosure of RS6077.[2]

The promising inhibitor was evaluated in cancer cell culture and in a lymphoma TMD8 mouse xenograft model. Compound **1** showed a strong cell cycle arresting potential, blocking or dramatically delaying mitosis. In HeLa cultures, the cell cycle is strongly delayed prior to reaching mitosis. The mitotic arrest induced in RPE-1 non-transformed cells is not necessarily followed by cell death induction. In AHH-1 cell cultures **1** causes effective mitotic block suggesting that death actually occurs during mitotic arrest in cells that fail to complete mitosis. Compound **1** inhibits different lymphoma cell lines at nanomolar concentration (figure 1). Most importantly, exposure to the different doses of **1** does not change cell viability of healthy cells, thus indicating this compound is not toxic for normal cells. In the lymphoma TMD8 mouse xenograft model, **1** reduces the tumor size after administration in 30% PEG400 by oral gavage at 100 mg/kg. These findings indicate that **1** has potential as novel therapeutic agent to treat lymphoma cancers.

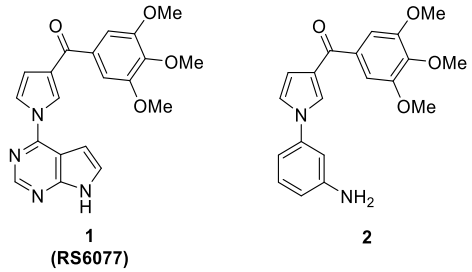


**Chart 1.** Chemical structures of compound **2** and **3**

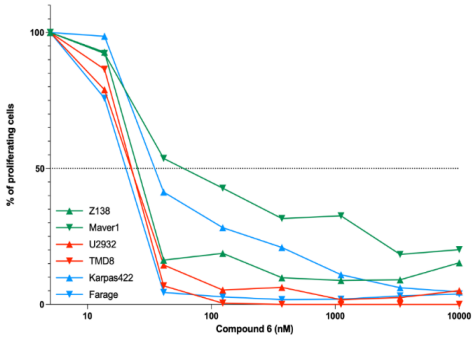


**Figure 1.** Inhibition of proliferation by compound **1** in lymphoma cells.

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PO-o84

# DISCOVERY AND OPTIMIZATION OF INDOLINE-BASED COMPOUNDS AS DUAL 5-LOX/SHE INHIBITORS: IN VITRO AND IN VIVO ANTI-INFLAMMATORY CHARACTERIZATION

Smaldone, G.; [a] Cerqua, I.; [b] Musella, S.; [a] Peltner, L. K.; [c] D'Avino, D.; [b] Di Sarno, V.; [a] Granato, E.; [b] Vestuto, V.; [a]Di Matteo, R.; [b] Pace, S.; [c] Ciaglia, T.; [a] Bilancia, R.; [b,c] Di Matteo, F.; [a] Di Micco, S.; [d] Bifulco, G.; [a] Gomez-Monterrey, I.; [b] Campiglia, P.; [a,d] Ostacolo, C.; [b] Roviezzo, F.; [b] Werz, O.; [c] Rossi, A.; [b] Bertamino, A.[a]

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Multi-target drugs have attracted considerable interest over the past decade for their advantages in treating complex diseases and health conditions; in particular, this approach seems suitable in the field of anti-inflammatory drugs for avoiding shunting and/or redirection phenomena. [1,2,3] In this context, 5-LOX/sEH dual inhibitors offer the advantage of blocking the pro-inflammatory LT production and simultaneously increasing the anti-inflammatory eicosanoid levels. [4,5] In the present work we describe the synthesis and pharmacological evaluation of a new series of compounds, designed as 5-lipoxigenase (5-LOX) inhibitors. An *in silico* analysis of an in-house library led to the selection of nine compounds as potential 5-LOX inhibitors; the enzymatic and cellular assays showed the indoline-based derivative **43** as a notable 5-LOX inhibitor, guiding the design of a library of analogues. The next *in vitro* investigation of the new series led to the identification of dual 5-LOX/sEH inhibitors with **73** showing the most promising activity ($IC_{50}$s of 0.41 ± 0.01 and 0.43 ± 0.10 µM for 5-LOX and sEH, respectively); Compound **73** exhibits a remarkable anti-inflammatory efficacy in two different murine models of inflammation: zymosan-induced peritonitis and ovalbumin induced asthma. These results are an interesting starting point for the design of 5-LOX/sEH dual inhibitors and for the further investigation of their use as anti-inflammatory agents.

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PO-085

# POTENTIAL COVID-19 THERAPIES FROM IN-SILICO REPURPOSING OF DRUGS AGAINST THE SARS-COV-2 HELICASE

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FDA (Food and Drug Administration) approved drugs for human use and investigational molecules were evaluated as potential inhibitors of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In particular, we focused our attention on SARS-CoV-2 helicase (NSP13) due to its ability to catalyse the unwinding of double-stranded DNA or RNA in a 5' to 3' direction and to act in concert with the replication-transcription complex (NSP7/NSP8/NSP12). In this work, we performed a detailed conservation analysis of SARS-CoV-2 helicase and we applied computational and bioinformatics tools in order to predict the enzyme binding pockets adopted for an *in silico* drug repurposing approach. After the identification of 4 SARS-CoV-2 binding pockets (**Figure 1**), a structure-based virtual screening was performed with the aim to find promising compounds with a multi-site profile.

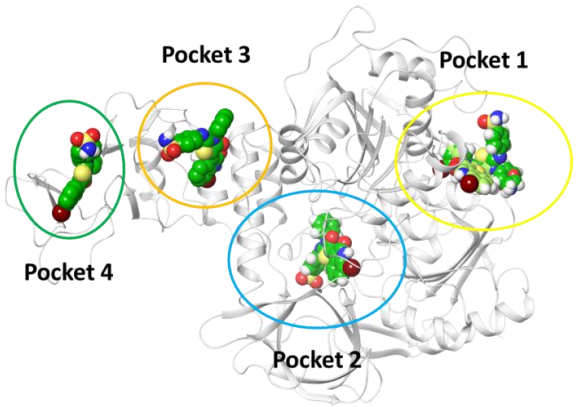


**Figure 1.** 3D representation of the 4 SARS-CoV-2 helicase (PDB code 7NN0)[1] binding pockets.

According to their G-score ranking, we chose 15 shared compounds able to recognize 3 out of 4 sites. Finally, by visual inspection and based on their commercial availability, we purchased 6 promising compounds to test their enzymatic activity. Among them, two investigational compounds were found able to inhibit the enzymatic activity in a micromolar range.

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PO-o86

# 5-HT$_{1A}$ RECEPTOR LIGANDS AS VERSATILE TOOLS FOR CNS DISORDERS AND PAIN

Sorbi, C.;[a] Linciano, P.;[a] Lesniak, A.;[b] Bujalska-Zadrożny, M.;[b] Pawłowska, A.;[b] Bielenica A.;[c] Orzelska-Gorka, J.;[d] Kędzierska E.;[d] Biała, G.;[d] Ronsisvalle, S.;[e] Denora, N.;[f] Limoncella, S.;[g] Casarini, L.;[g] Cichero, E.;[h] Fossa, P.;[h] Satała, G.;[i] Bojarski A.J.;[i] Bardoni, R.;[j] Brasili, L.;[a] Franchini, S.[a]

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The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) subtype has long been studied as a relevant pharmacological target for the treatment of anxiety and depression. More recently, 5-HT$_{1A}$R ligands have been also proposed for cognitive impairment, Parkinson's disease (PD), schizophrenia, pain, and cancer therapy. Over the years, extensive SAR studies, described by our research group, demonstrated that the 1,3-dioxolane moiety is a versatile scaffold for potent and selective 5-HT$_{1A}$R ligands. The expansion, opening and simplification of the 1,3-dioxolane ring was also explored and the 1,3-dioxane derivative **1** emerged as a potent and selective ($pK_i$= 8.8; $pD_2$= 9.22; $\%E_{max}$= 92) full 5-HT$_{1A}$R agonist. Pharmacokinetic analysis revealed its high brain distribution and the behavioral studies demonstrated its anxiolytic and anti-depressant effects.[1] Successively, a new series of 2-heteroaryl-phenoxyethylamines was synthesized and the 4-pyridyl derivative **2** resulted a potent and selective 5-HT$_{1A}$R partial agonist. The role of chirality in the interaction with 5-HT$_{1A}$R was evaluated: no significant enantioselectivity was seen in the binding experiments and the docking studies supported the biological data. Then compound **2** was tested *in vivo* (mice) in both acute (hot plate test) and severe tonic (intraplantar formalin test) pain models and its effect was reverted by WAY-100635. Moreover, to exclude any interaction with opioid receptors, electrophysiological studies were performed in the presence of naloxone. The results showed no significant differences for both outward currents and spontaneous excitatory postsynaptic current frequency, thus excluding any interaction with the opioid receptors. These findings suggest that **2** could be a good candidate for acute and chronic pain therapy since it lacks the adverse effects commonly associated with classical opioids.[2]
It has been described that phenoxyethylamine fragment might play a role in the interaction with dopamine receptor. Thus, the racemate **2** and the two enantiomers were also tested for binding affinity at $D_2$ receptors. Interestingly, unlike 5-HT$_{1A}$R, a marked enantioselectivity was seen, with **(*S*)-2** being 100-fold more potent than the corresponding R isomer and behaving as a full $D_2$ antagonist. It is known that a multitarget therapeutic approach, i.e. combining the $D_2$ antagonism and 5-HT$_{1A}$ agonism, could be beneficial in case of multifactorial pathologies such as schizophrenia. In a preliminary *in vivo* study **(*S*)-2** was able to counteract the amphetamine induced hyperactivity, thus highlighting its potential in the treatment of this disorder. Moreover **(*S*)-2** showed promising developability features such as high selectivity over other 5-HT subtypes (5-HT$_{2A/2C/6/7}$), no interaction with μ receptors, and low hepato/cardiotoxicity that make this compound a valuable preclinical candidate for CNS disorders and pain therapy.

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PO-o87

# BERBERINE STRUCTURE SIMPLIFICATION: SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIBACTERIAL ANALOGUES

Rotondo, N.P.;[a] Milani, G.;[a] Di Somma, A.;[b] Quintieri, L.;[c] Caputo, L.;[c] Duilio, A.;[b] Cavalluzzi, M.M.[a]; Lentini, G.[a]

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Berberine, the main bioactive component of many medicinal plants belonging to *Berberidaceae* and *Rutaceae* families[1], is endowed with several pharmacological properties including broad antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria[2]. With the aim of overcoming some berberine drawbacks such as low oral bioavailability, high MIC values, low fraction of $sp^3$ carbon atoms and flatness, the molecular simplification approach was applied to the secondary metabolite and a series of more flexible open models of berberine were prepared (Figure 1) and screened for their antimicrobial activity against Gram-positive and Gram-negative bacteria. Furthermore, since berberine can inhibit the filamentous temperature-sensitive Z protein (FtsZ)[3], a bacterial protein involved in cell division, a 3D quantitative structure–activity relationship (3D-QSAR) study on this target has also been performed. Rewardingly, some of the berberine simplified analogues displayed higher potency than the parent compound, in good agreement with molecular docking simulation prediction.[4]

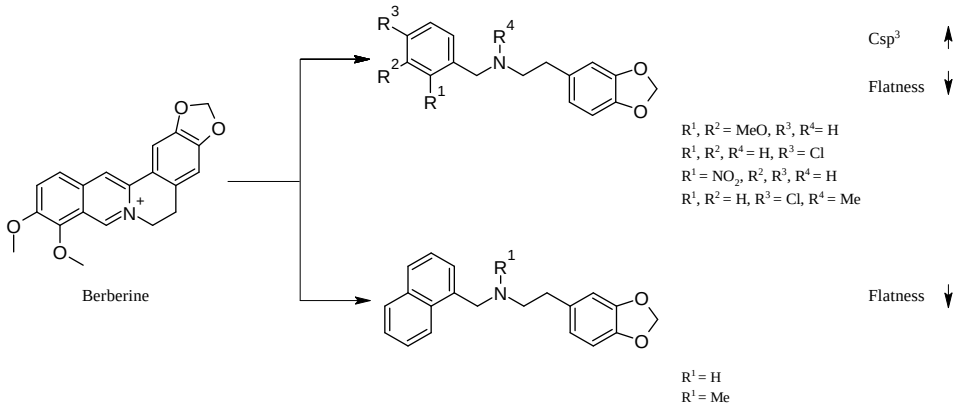


**Figure 1**

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PO-o88

# DESIGN AND SYNTHESIS OF NEW CK1δ PROTEIN KINASE INHIBITORS

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Casein kinase (CK) is a type of conserved serine/threonine protein kinase that phosphorylates many important proteins. The phosphorylation process catalysed by these kinase has the purpose of modulating substrate proteins through alterations that can lead to an increase or a decrease in their intrinsic activity. This process is of particular importance in the transduction pathways of intracellular signals. There are two families of casein kinase: CK1 and CK2 and in this thesis we have dealt in particular with the family of protein kinases CK1, of which, in mammals, seven isoforms have been identified: CK1α, CK1β, CK1γ1, CK1γ2, CK1γ3, CK1δ e CK1ε. CK1 is omnipresent and is involved in the regulation of several biological functions: membrane transport, circadian rhythm, p53 and mdm2 phosphorylation, cell division, apoptosis, Wnt signalling pathway. The activity of CK1 is involved in the evolution of some disease, in particular CK1δ is closely related to neurodegenerative diseases such as Alzheimer's and Parkinson's. In particular, in the brain of Alzheimer's patients, CK1δ isoform is present in high concentrations evoking a hyper-phosphorylation of the tau protein, causing its dissociation from the microtubules, with consequent their destabilization and inducing neuronal death. Furthermore, the CK1δ and other isoforms appear to be involved in the formation of Lewy bodies thus demonstrating a possible involvement also in the development of Parkinson's disease.[1]

The aim of this work was to obtain new of CK1δ inhibitors endowed with purine scaffold potentially able to interact at the ATP-binding site of the enzyme. Therefore, in a preliminary evaluation of some in-house compounds, the adenine bearing a cyclopentyl ring in 9 position was found able to inhibit the enzyme up to 61% of its activity, when tested at 40 µM. Hence, a new series of 9-substituted adenine derivatives (Figure 1) were designed and synthetized starting from the commercially available 2,6-dichloropurine introducing different substituents in 2, 6 and 9 positions.

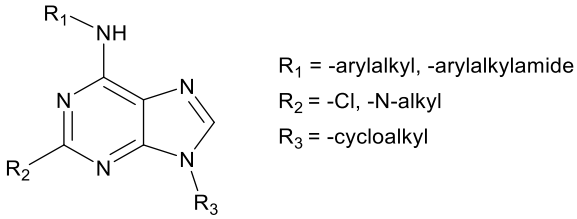


**Figure 1.** New designed CK1δ antagonists

All the derivatives were tested to evaluate their ability to inhibit the enzymatic activity at fixed concentration of 40 µM, the compounds that left a residual kinase activity less than 50% were also tested at 10 µM and, then, $IC_{50}$ was determined in case the residual enzymatic activity was less than 50%.
The new derivatives were found to be inhibitors of the CK1δ enzyme with activities reaching up to 99%.

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PO-o89

# MOLECULAR PROBES TO DISSECTING 34k-BIOLOGY: SPR-AIDED FRAGMENT-BASED ROUTE TO POTENTIAL BINDERS

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The spread of tiger mosquito Aedes albopictus as vector responsible for the transmission of several arboviral diseases like Zika, yellow fever and dengue, has enormously increased over the last decades worldwide,[1] resulting in high social and financial costs on public health. Given the lack of specific antiviral treatments or effective vaccines, vector monitoring and control along with prevention of mosquito bites still represent the main tools to contain arboviral disease transmission.[2] Thus, the need of finding new strategies for developing potential therapies and/or stopping the pathologies transmission is urgent. In this scenario, 34k- insect's salivary protein family, and in particular Aedes albopictus 34k-2 isoform, playing a key role as secondary messenger for host-target identification in the insect-blood feeding process, has been proved to increase both viral infectivity in the host[3] and contacts between human cells and virus.[4] Therefore, blocking these signals may disrupt the probing mechanism, thus decreasing the female mosquito ability to acquire a blood meal and then reproduce. This, ultimately, leads to a reduction of the mosquito population and to a decline of arboviral disease transmission to humans. Aided by the knowledge of the 34k-proteins X-ray crystal structure along with the availability of optimized protein expression and purification techniques, a fragment-based drug-design (FBDD) approach was employed aiming at seeking 34k-potential binders able to modulate the activity of these proteins or to assess their biological function. A small library of molecular fragments was designed and, subsequently, submitted to biophysical screening through surface plasmon resonance (SPR) experiments to identify potential 34k-ligands as displayed in **Figure 1**. False positive removal and kinetics analysis returned as a potential binder compound C1 (substituted pyruvic acid, $K_D$ = 1170 μM, LE = 0.27).

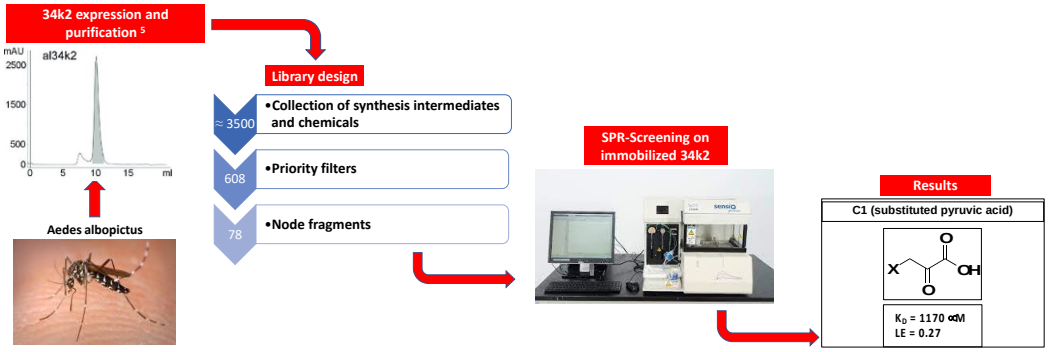


**Figure 1.** Fragment-based approach workflow.

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# REPURPOSING OF METHYLPHENIDATE ANALOGUES AS PARKINSON'S DISEASE-MODIFYING AGENTS: DESIGN; SYNTHESIS AND THEIR IN VITRO AND IN VIVO EVALUATION

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Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by a strong dopaminergic nigrostriatal neurons degeneration and the presence of Lewy bodies, mainly composed of α-synuclein (αSyn) fibrillary aggregates. It has been recently demonstrated how the neuronal phosphoprotein Synapsin III (Syn III) participates in αSyn pathology in PD brains and is a direct player of αSyn aggregation[1]. We recently described that the monoamine reuptake inhibitor methylphenidate (MPH) let the motor activity recovery of human αSyn tg mice, following a dopamine transporter (DAT)-independent mechanism[2]. MPH let the re-establishment of the functional interaction between Syn III and α-helical αSyn, suggesting that the pathological αSyn/Syn III interaction could constitute a therapeutic target for PD[2].

In this communication, we present our updates on the development of two generations of MPH analogues as modulators of the pathological αSyn/Syn III interaction[3]. We started with the preparation of a "first generation" series of compounds, in which we introduced small to hindered substituents on the aromatic moiety of MPH. We evaluated their ability to stimulate *in vitro* the functional interaction between α-Syn and Syn III and a first lead candidate emerged. PK1 showed improved capacity of modulating αSyn/Syn III interaction and owned the ability to reduce αSyn aggregation *in vitro* and to restore the motility of αSyn tg mice *in vivo* more efficiently than MPH[4].

Then, driven by a proper developed computational model, we prepared a "second generation" of compounds[5], where we changed the distance between the piperidine and the phenyl moieties, and we further introduced different substituents in *para* position of the aromatic ring. PK7 and PK12 proved their disease-modifying effect, by inducing the interaction between αSyn and Syn III and by reducing αSyn aggregation *in vitro*. Furthermore, they completely lost off-targets effect on DAT and on other MATs, thus avoiding any side effect, common of MPH derived molecules.

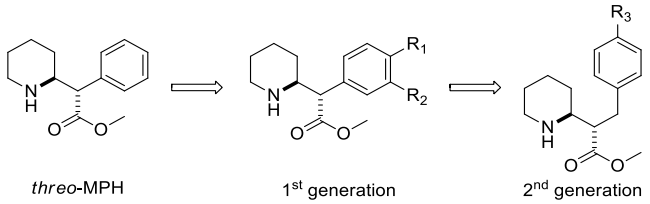


**Figure 1:** Structures of methylphenidate (MPH) and MPH analogues, object of the present communication.

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5. Unpublished data

PO-091

# NOVEL PEPTIDOMIMETIC INHIBITORS OF BLOOD COAGULATION FACTORS WITH POTENTIAL AGAINST SARS-CoV-2 INFECTION

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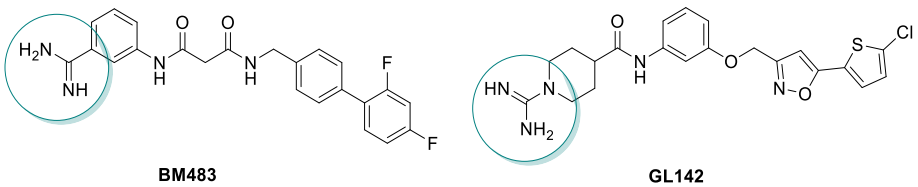
The SARS-CoV-2 pandemic prompted the scientific community to unprecedented efforts in the attempt to identify new targets and therapeutic approaches. Many research projects are ongoing worldwide for discovering new druggable targets and drugs to fight SARS-CoV-2 infection, which can result in high-rate incidence of fatal disseminated intravascular coagulation (DIC) and pulmonary thromboembolism. Some observational studies suggested that direct oral anticoagulants (DOACs) may protect patients with COVID-19 showing atrial fibrillation.[1]

The blood coagulation factor Xa (fXa) and thrombin (thr) are widely expressed in pulmonary tissues. They share high structural homology with TMPRSS2, a human serine protease that cleaves and primes the viral Spike protein leading to fusion between the viral envelope and the host cell membrane.[2] Moreover, nafamostat, a guanidine/amidine containing antithrombin agent, has been identified as a covalent TMPRSS2 inhibitor.[3]

With the aim of developing a ligand acting both at the early stage of the infection (cell entry) and downstream of the SARS-CoV-2 induced thrombotic complications[4], we investigated highly potent fXa-selective inhibitors built up on two different peptidomimetic scaffolds, namely the isonipecotamides (e.g., **GL142**) and the newly synthesized malondiamides (e.g., **BM483**), both bearing a basic guanidine or benzamidine function (Figure 1). Tested on *in vitro* SARS-CoV-2 infection models, some of them resulted moderately effective in suppressing viral replication in VERO E6 cells. Structure-activity relationships and Molecular Docking calculations highlighted molecular determinants that may help to understand the key interactions with TMPRSS2.




BM483 GL142

**Figure 1**

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PO-092

# NEW IFENPRODIL-BASED ANALOGUES AS NEUROPROTECTIVE AGENTS

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NMDA receptors are heterotetrameric glutamatergic ionotropic receptors. They have an important role in several CNS functions, as well as in neuronal excitotoxicity, often associated with many diseases such as Alzheimer, Parkinson, and others. In this regard, GluN2B-containg NMDA receptor antagonists are of interest for the treatment of CNS disorders. On the other hand, sigma-1 receptor subtype (σ1R), plays a fundamental role in the regulation of NMDA receptors and display neuroprotective effects through many different pathways.[1]

Ifenprodil (**1**, Figure 1)[2] is one of the prototypical allosteric inhibitors which interacts only with the GluN2b-containing NMDARs[3] (Ki = 10 nM), but it has a strong correlation with the typical chemical features belonging to the common σ1R ligands, showing however a moderate affinity for this receptor subtype (Ki = 125 nM).

Aiming to discovery new selective and potent σ1R entities, and concurrently discover novel GluN2bR ligands, we designed and synthesized new molecules correlated to ifenprodil, obtained by retaining the phenylpropanone motif and by jointly replacing the 4-benzylpiperidine fragment with other amine moieties present in some well-known σ1R ligands. Some representative compounds of the series have been chosen for a computational evaluation in order to understand the chemical interactions with the active binding site of the proteins.

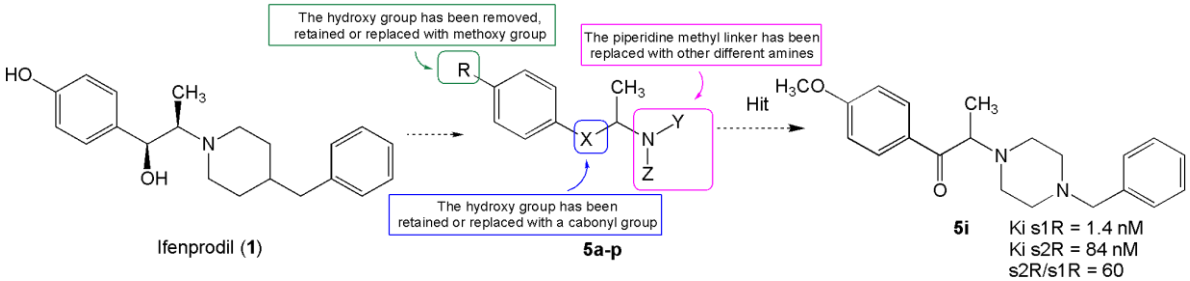


**Figure 1.** Rationale of the new ifenprodil analogues **5a-p**

The synthesized compounds were evaluated for affinity to both σ1R and σ2R, through radioligand binding assay and three out of sixteen derivatives showed Kiσ1R value less than 10 nM. The best compound of the series resulted the piperazine-based derivative **5i** with Kiσ1 = 1.4 nM and the best selective profile with a 60-fold preference for the σ1R over σ2R. Binding evaluation towards GluN2b receptor and neuroprotection assays are still ongoing.

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PO-093

# DEVELOPMENT OF POTENT FORMYL PEPTIDE RECEPTOR 1 (FPR1) ANTAGONISTS WITH ISOFLAVONE STRUCTURE

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The Formyl Peptide Receptor 1 (FPR1) is a member of the chemotactic formyl peptide receptor family, whose primary function is the trafficking of various leukocytes into sites of bacterial infection and inflammation.[1] Recent studies have documented the expression of FPR1 in different types of cancers, including glioblastoma, neuroblastoma, colon, breast, and bladder cancer. FPR1 plays a significant role in expansion, resistance, and recurrence in the cancer context. Several studies have reported that FPR1 antagonists can significantly reduce the proliferation and invasion of tumor cells both in vitro and in vivo, thus providing new avenues for cancer treatment.[2]

We recently identified the isoflavone FPR1 antagonist **1** (Figure 1) with promising antagonist activity in the submicromolar range ($IC_{50}$ = 0.31 μM).[3] Therefore, we began a medicinal chemistry campaign to increase the FPR1 antagonist potency further. The structural modifications of **1** regarded the 2-, 3-, 6-, and 7-position of the isoflavone nucleus, leading to the identification of the antagonist **2** (Figure 1), which has potency in the nanomolar range ($IC_{50}$ = 25 nM).

We will report on the design, synthesis, and biological characterization efforts that led to the identification of compound **2**.

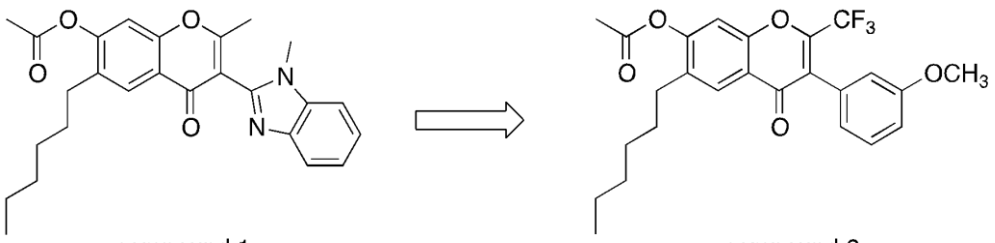


**Figure 1.** Isoflavone FPR1 antagonists

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PO-094

# IDENTIFICATION OF A NOVEL BIOACTIVE TETRAPEPTIDE DERIVED FROM SPIRULINA PLATENSIS

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*Arthrospira platensis*, known as spirulina, a filamentous fresh-water planktonic cyanobacterium, has an enhanced nutritional profile with high bioavailability of essential amino acids, biliproteins and other pigments, B and E vitamins, mineral substances and trace elements, glycolipids, sulpholipids, and essential polyunsaturated fatty acid. They provide therapeutic properties in the treatment and prevention of a variety of disorders.[1] Interestingly, Spirulina has been reported to exert biological activities and have beneficial properties in the management of cardiovascular diseases.[2]

In a recent work, through a peptidomic analysis of a spirulina formulation subjected to gastrointestinal digestion, we identified a decameric peptide, GIVAGDVTPI, named SP6 (*Spirulina Peptide 6*). In vivo administration of SP6 reduced blood pressure, improved endothelial vasorelaxation, and exerted an antihypertensive action in experimental models of hypertension, working through a NO-dependent mechanism.[3]

Considering the above highlighted, the aim of this study was to elucidate structure-activity relationships of SP6 in order to identify the primary sequence of the peptide, we synthesized a library of overlapping peptides, with specific length and specific offset covering the entire SP6 sequence. The synthetized peptides were evaluated for their ability to modulate vascular function. Results obtained led to the identification of an interesting tetrapeptide that show the same activity of the parent peptide.

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PO-095

# DEVELOPMENT AND APPLICATION OF A BIOPHYSICAL PLATFORM FOR THE IDENTIFICATION OF FRAGMENTS FOR THE DEVELOPMENT OF SETD8 LIGANDS

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SETD8/SET8/Pr-SET7/KMT5A is the only known protein lysine methyltransferase (PKMT) that catalyses the monomethylation of histone H4 Lys20 (H4K20me1). Besides H4K20, SETD8 methylates lysine residues of many other proteins, such as the proliferating cell nuclear antigen (PCNA) and the tumour suppressor p53.[1] For this reason, a dysregulation of SETD8 is related to different pathological conditions, including cancer.[2,3] Despite the steadily growing interest in physiological and pathological roles of SETD8, to date only few selective inhibitors of this protein have been reported.
Here we report the development and the optimization of a method for the screening of a fragment library aimed to the identification of new chemical scaffolds, that can be used to develop potent and selective inhibitors of SETD8. We applied a fragment-based drug discovery (FBDD) approach (Figure 1), consisting of preliminary hits identification by Differential Scanning Fluorimetry (DSF), and then hits validation by Surface Plasmon Resonance (SPR). The low chemical complexity of the identified fragments will allow a more efficient exploration of chemical space and the information of their binding properties will be used to guide their optimization using medicinal chemistry strategies.

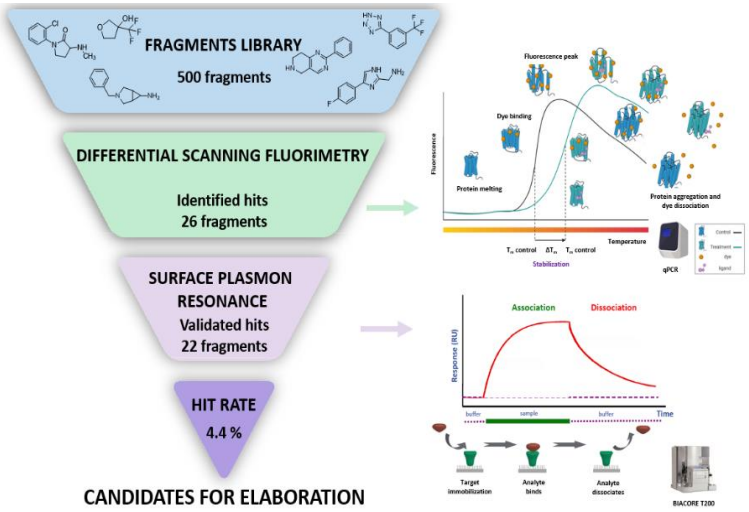


**Figure 1 Fragment-based drug discovery (FBDD) approach.**

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PO-096

# COX-1 INHIBITORS AS ANTI-PLATELET AGENTS IN COVID-19

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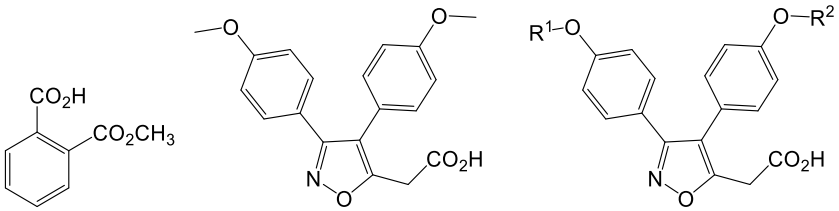
Coronavirus Disease 19 (COVID-19) is primarily a lung disease which frequently lead to major cardiovascular complications and a poor prognosis due to excessive platelet activation, uncontrolled immune/inflammatory reactions ("cytokine storm"), endothelial dysfunction, and coagulopathy.[1] Aspirin, due to its anti-inflammatory and anti-platelet aggregation properties, has been evaluated as a potential therapeutic agent for COVID-19. Low-doses Aspirin (typically 75–81 mg/day) irreversibly inhibits platelet cyclooxygenase-1 (COX-1) by $Ser_{530}$ acetylation preventing conversion of arachidonic acid into $PGG_2/PGH_2$, the latter in turn transformed by thromboxane synthase in thromboxane $A_2$, thus resulting in antithrombotic effect. Unfortunately, its use is limited by gastrointestinal side effects and aspirin resistance.[2] Therefore, novel COX-1 inhibitors are needed. Mofezolac (Figure) is the most potent and selective COX-1 inhibitor administrated to humans as an anti-arthritis drug (Disopain™). It belongs to the diarylisoxazoles chemical class and used as "hit compound" for Structure Activity Relationship (SAR) studies to design novel leads with antithrombotic activity. Replacing one or both mofezolac methoxyls by chemical groups with either increasing steric hindrance and capable to establish different interactions inside COX-1 active site allowed the identification of novel COX-1 inhibitors. Evaluation of their effects on blood coagulation cascade is ongoing.




**Aspirin** **Mofezolac** **Mofezolac analogues**

$R^1$, $R^2$ = Alkyl, Aryl

**Figure.** Chemical structures of aspirin, mofezolac and, mofezolac analogues.

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PO-097

# HDAC 6 INHIBITORS WITH A NEW 3-HYDROXY-ISOXAZOLE ZINC BINDING GROUP

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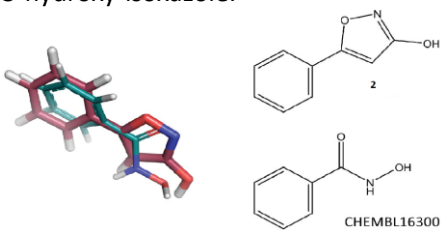
Histone deacetylase 6 (HDAC6) heavily impact on tumor cells invasion and metastasis, consequently is a well-established drug target for cancer treatment. The clinical relevance of HDAC inhibitors (HDACi) is proven by the presence of five drugs and several clinical candidates.[1] The great majority of the developed inhibitors contains a hydroxamic acid zinc binding group (ZBG), but several studies have shown that hydroxamic acids are genotoxic.[2] This undesirable effect motivates the search for HDACi with alternative ZBGs. Recently we described the effect of different linker chemotypes on the potency and selectivity of a series of HDACi carrying a hydroxamate ZBG.[3] Interestingly, the N-hydroxy-3-phenyl-propiolamide **1** converted into the more stable 5-aryl-3-hydroxy-isoxazole **2**, presumably formed through intramolecular cyclization. To evaluate whether compound **2** could provide a valuable bioisosteric replacement of previously reported HDAC6 ZBGs, extensive ligand-based similarity analyses were performed in the ChEMBL database. The visual inspection of the predicted ligand alignments showed that compound **2** overlaps very well with the benzhydroxamic acid inhibitor ChEMBL16300 ($IC_{50}$=115 nM), resulting in a similar location of the two phenyl rings and a good alignment of hydrogen bond donor/acceptor groups of the hydroxamic acid and the 3-hydroxy-isoxazole.




Therefore, in this work a series of 3-hydroxy-isoxazole derivatives, bearing different aromatic or heteroaromatic linkers and various cap groups, was synthesized and, with regard to the SAR, docking studies were also performed to investigate their binding mode. They were tested *in vitro* for the inhibition of recombinant human HDAC6, through dose-response assays in the 135 nM to 300 µM concentration range. Some compounds were able to inhibit HDAC6 with good potency and the best candidate reached an $IC_{50}$ value of 700 nM. Moreover, for the most active inhibitors, we evaluated the effect on the histone H3 and α-tubulin acetylation levels together with the anti-proliferative activity in DU145 cell line. DU145 are androgen insensitive prostate cancer (PCa) cells with very low or undetectable AR levels that represents the castration-resistant PCa (CRPC) model. HDACs contribution to CRPC is known and the possibility to arrest CRPC proliferation by acting on HDAC targets is expanding in the scientific literature. In conclusion, this completely new 3-hydroxy-isoxazole ZBG associated to the good biological results that will be presented and discussed make this new series of HDAC6i particularly attractive.[4]

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PO-o98

# AN ALTERNATIVE *IN SILICO* APPROACH FOR PREDICTING DEVELOPMENTAL TOXICITY BASED ON EXPLAINABLE ARTIFICIAL INTELLIGENCE

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QSAR models have gained importance especially in predictive toxicology field such as DevTox: Developmental Toxicity[1]. In the last decades Artificial Intelligence achieved an increasing important strategy for toxicology prediction endpoints[2]. This work suggests a new *in silico* approach to explain a challenging health-human hazard. The proposed framework favorably compares with the state-of-the-art approaches in terms of accuracy, sensitivity and specificity, thus resulting in a reliable support system for developmental toxicity.

In this work, we employed the established CAESAR[3] training set made of 234 chemicals for model learning. Two test sets, including as a whole 585 chemicals, were instead used for validation and generalization purposes.

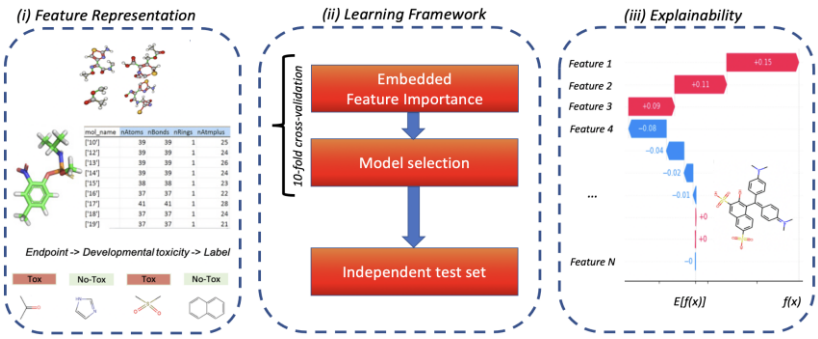


**Figure 1.** (i) input matrix are computed based on 818 descriptors. (ii) schematic pipeline to build and assert model. (iii) attempted strategy to comprehend the insurgent cause of DevTox.

We carried out a cross-validation analysis on the training-set to assess if the pool of the 818 calculated descriptors represents a reliable base of knowledge for developmental toxicity among a variety of classifiers. More specifically, we iterated a 10-fold cross-validation 100 times.

In terms of accuracy, all values observed are comparable in CV. Then, based on sensitivity and specificity values, XGB resulted as the best trade-off. SVM classifier provided the benchmark performance, instead. Our approach indicates that chemical descriptors play a key role in agreement to the explainability paradigm.

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PO-099

# NEW TARGETS AND INTERFERENTS TO FIGHT *STAPHILOCOCCUS AUREUS* RESISTANCE

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Human hemoglobin (Hb) is the preferred iron source of *Staphylococcus aureus*. This pathogenic bacterium exploits a sophisticated protein machinery called Iron-regulated surface determinant (Isd) system to bind Hb, extract and internalize the heme and finally degrade it to complete iron acquisition, so escaping the host nutritional immunity (NI). In particular, IsdB is a proven virulence factor of *S. aureus* and has been the object of so far ineffectual vaccine design strategies.

We set up a multidisciplinary platform to characterize IsdB-mediated iron acquisition and test small molecules able to inhibit this process, restore the natural NI and inhibit bacterial infection. The stoichiometry, mechanism and kinetics of IsdB:Hb complex formation were investigated by absorption spectroscopy, surface plasmon resonance (SPR) and molecular dynamics simulations, and site-directed mutagenesis was exploited to validate the role of key residues for heme binding and extraction.[1] By single-particle cryo-EM we determined the structure of two key intermediate species along the process,[2] and by means of accelerated molecular dynamics techniques we are studying the mechanism of heme transfer, the key residues involved and the associated free energy.

A Structure-Based Virtual Screening (SBVS) campaign was carried out to identify small molecules able to disrupt IsdB:Hb protein-protein interaction (PPI), and an in-house developed ELISA test was used to screen a set of commercially available compounds. STD-NMR was applied to verify specific interactions of a sub-set of molecules, chosen on the basis of their ability to reduce the amount of Hb bound to IsdB. Direct binding was verified for 3 of these, that were *de novo* synthesized and validated by ELISA and STD-NMR. Crystal structures of Hb in complex with one of these compounds have been obtained and will drive Structure-Activity Relationship studies for compound optimization.

To our knowledge, this is the first attempt to target by small molecule ligands the interaction between Hb and IsdB. A platform is under development for testing more potent inhibitors that will be identified, *in vitro* and in *S. aureus* cell cultures (PRIN2020 2020AE3LTA "ERASE: dEfeat antimicrobial ResistAnce through iron starvation in *Staphylococcus aureus*").

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PO-100

# THE EFFECTIVENESS OF GLIFLOZINS IN HEART FAILURE: AN EXPLAINABLE ARTIFICIAL INTELLIGENCE APPROACH

Mele, M.;[a] Togo, M.V.;[b] Mele, A.;[b] Imbrici, P.;[b] Nicolotti, O.;[b] Brunetti, N.D.;[c] Correale, M.;[a] De Luca, A.;[b] Liantonio, A.;[b] Altomare, C.D.[b] and Amoroso, N.[b,d]

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Recent cohort-based studies have demonstrated the effectiveness of sodium-glucose cotransporter 2 (SGLT2) inhibitors gliflozins for heart failure[1,2]. However, to what extent this effectiveness impacts on patients' clinical conditions and instrumental variables remains unknown. Here, we evaluated the effects of gliflozins in a cohort of 78 consecutive diabetic patients enrolled at Policlinico Riuniti University Hospital in Foggia. Patients treated with gliflozins can be accurately distinguished (5-fold cross-validation accuracy of 71 ± 11 %) from patients undergoing standard treatment for heart failure especially thanks to echocardiographic evaluations measured at two different time points (baseline and 3-6 months follow-up). In addition, using an eXplainable Artificial Intelligence framework based on the Shapley paradigm[3], we characterized patients treated with gliflozins but not showing a significant clinical improvement, see Figure 1.

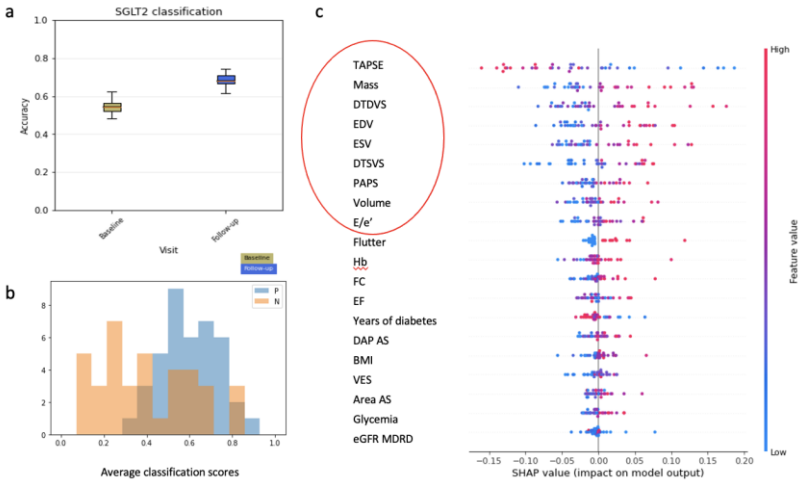


**Figure 1.** The use of gliflozins improves patients' clinical conditions so that at follow-up it was possible to accurately distinguish the treated patients from patients treated with alternative therapies (panel a). However, cross-validation analyses showed the presence of misclassified patients, not-responding to gliflozins (panel b). We ranked the features driving this behavior according to the Shapley paradigm (panel c).

High values of Tricuspid Annulus Plane Systolic Excursion (TAPSE), an index of right ventricle systolic function, characterize patients responding to gliflozins, while lower values weakened its effectiveness. Analogous considerations could arise for the other variable. Low values of left ventricle mass (Mass), left ventricle end-diastolic diameter (DTDVS), left ventricle end-diastolic and end-systolic volume (EDV, ESV) suggest the possibility for gliflozins to be more effective.

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PO-101

# FROM AN IN-HOUSE LIBRARY OF 2-PHENYLQUINOLINES TO BROAD-SPECTRUM ANTI-CORONAVIRUS AGENTS

Nizi, M.G.;[a] Persoons, L.;[b] Corona, A.;[c] Felicetti, T.;[a] Cernicchi, G.;[a] Massari, S.;[a] Manfroni, G.;[a] Vangeel, L.;[b] Barreca, M.L.;[a] Esposito, F.;[c] Jochmans, D.;[b] Milia, J.;[c] Cecchetti, V.;[a] Schols, D.;[b] Neyts, J.;[b] Tramontano, E.;[c] Sabatini, S.;[a] De Jonghe, S.;[b] Tabarrini, O.[a]

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A selection of compounds from a proprietary library, based on chemical diversity and various biological activities, was evaluated as potential inhibitors of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a phenotypic-based screening assay. Among the 300 tested compounds, a derivative based on a 2-phenylquinoline scaffold[1] emerged as the most promising hit with an $EC_{50}$ and $CC_{50}$ value of 6 and 18 µM, respectively. The subsequent selection of additional analogues, along with the synthesis of ad-hoc derivatives, led to compounds that maintained low µM activity as inhibitors of SARS-CoV-2 replication and lacked cytotoxicity up to 100 µM. In addition, the most promising congeners also show pronounced antiviral activity against the human coronaviruses HCoV-229E and HCoV-OC43, with $EC_{50}$ values ranging from 0.2 to 9.4 µM.[2]
Preliminary studies on the mechanism of action revealed that 2-PhQs were weak inhibitors of the autophagy pathway and were inactive as RdRp inhibitors, on the other hand some analogues inhibited the helicase unwinding activity of nsp13 with low µM potency. Nsp13 helicase is a very promising target, being highly conserved with a 99.8% sequence identity shared between SARS-CoV-2 and SARS-CoV-1, suggesting that drugs targeting nsp13 would be active also against emerging HCoVs outbreaks. For a few compounds, the helicase inhibition appears to mainly contribute to the antiviral activity, with bis-dimethoxyquinoline derivative that emerged as the most promising. The co-crystallographic experiments in progress for this compound, along with the synthesis of further analogues, will establish the importance of this group for nsp13 inhibition.

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PO-102

# TOWARDS A GREEN AND EFFECTIVE PRODUCTION OF BIOACTIVE SULPHATED STEROIDS USING HUMAN AND *DANIO RERIO* SULFOTRANSFERASE

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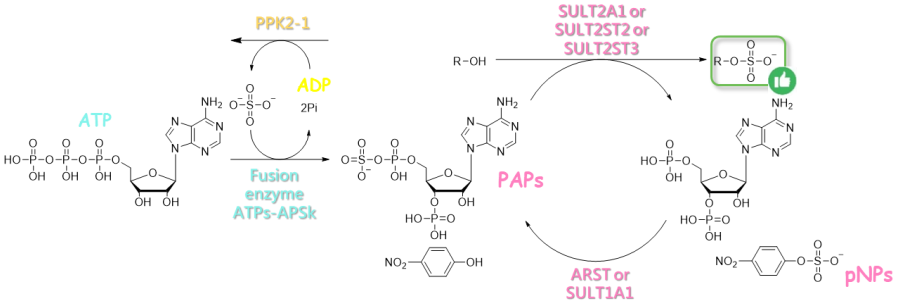
Steroids are widespread in nature, both in animal and plant kingdoms, and perform essential vital functions in higher organisms. The remarkable number of pharmaceutical applications make this class of compounds the second largest sector of the pharmaceutical industry after antibiotics, with a world market of around 10 billion dollars and a production exceeding 1,000,000 tons per year. [1] Steroidal active pharmaceutical ingredients (steroidal APIs) have been classically synthesized through chemical processes which often involve toxic reactants, harsh conditions, and possess mediocre selectivity. [2] Given the high production volume of these compounds and the increasingly restraining environmental regulations, new alternative methods are highly desirable. For instance, biocatalysis proved to be a useful tool in the development of greener, efficient, and selective processes. [3] Despite the important role played by steroidal sulphates in several physiological processes, [4] enzymatic sulphation is a very scarcely investigated field, since the PAPs cofactor's instability and its high cost make this transformation extremely challenging to be developed for synthetic purpose. [5] Taking inspiration from these considerations, in this communication, we report our ongoing efforts towards the development of a biocatalytic sulfation approach to be applied in steroid functionalization. The method has been accomplished under mild reaction conditions, and the cofactor's high cost and instability were overcome by an in-situ synthesis and regeneration from much cheaper and readily available starting materials. This work is part of a joined doctorate between the University of Perugia and the University of Amsterdam.




**Scheme 1.** Biocatalyzed sulphation of steroids and PAPs cofactor regeneration**.**

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PO-103

# NEW SULFONAMIDE INHIBITORS OF VIBRIO CHOLERAE CARBONIC ANHYDRASES: SYNTHESIS, ENZYME INHIBITION AND COMPUTATIONAL STUDIES

Tondo, A.R.;[a] Liguori, F.;[a] Fantacuzzi, M.;[a] Angeli, A.;[b] D'Agostino, I.;[a] Carta, F.;[b] Agamennone, M.;[a] Nicolotti, O.;[c] Carradori, S.[a] and Supuran, C.T.[b]

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Antimicrobial resistance (AMR) is a crucial issue estimated to cause 25,000 deaths per year. This evidence prompts intensive research in identifying alternative tools to be exploited in the fight against bacterial infections.[1] The selection of alternative macromolecular targets can help evade resistance, and bacterial carbonic anhydrases (CAs) can play an important role.[2] CAs are ubiquitous metalloenzymes responsible for the hydration of $CO_2$, an essential process in many organisms. In *Vibrio cholerae*, a Gram-negative pathogen, the $HCO_3^-$ concentration induces the expression of ToxT, a transcription factor involved in the pathogen's virulence. Therefore, the inhibition of *Vibrio* CAs could represent an alternative and useful tool to fight this pathogen.[3] Several sulfonamides have already been identified as *Vibrio* CA inhibitors.[4,5] In the present work, a library of 45 compounds clustered into three scaffolds (**Figure 1**) was synthesized exploiting a new synthetic approach and tested in enzyme inhibition assays against the two isoforms of *Vibrio* CAs (VchαCA, VchβCA), and the two human CAs (hCA I and hCA II) to assess their activity and selectivity. To explore the structural characteristics required for a better discrimination of less conserved regions of the enzyme, we also rigidified the linear linker into an imidazolidin-2-one, thus providing an additional point of H bond-mediated interaction with the target enzymes.

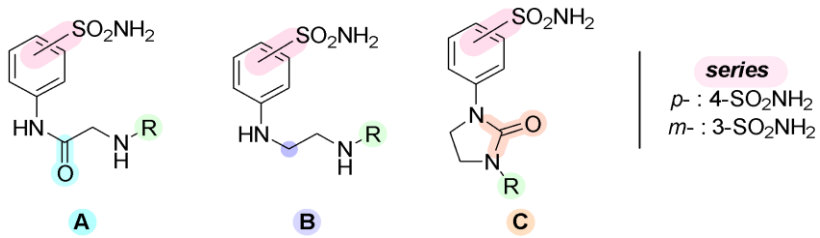


**Figure 1**. Chemical scaffolds of the new sulfonamides.

All compounds showed a nanomolar inhibition of *Vibrio* CAs, and some derivatives were endowed with good selectivity over human off-target isoforms. A computational study was conducted to depict the ligand's binding process and explain selectivity. The homology model of VchαCA was generated and used along with the 3D coordinates of VchβCA, hCA I, and hCA II for the subsequent structure-based studies.

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PO-104

# PLATO: A USER-FRIENDLY WEB PLATFORM FOR TARGET FISHING AND BIOACTIVITY PREDICTION

Trisciuzzi, D.;[a] Gambacorta, N.;[a] Tondo, A.;[a] Togo, M.V.;[a], Mastrolorito, F.;[a] Amoroso, N.;[b,c] Altomare, C.D.;[a] Ciriaco, F.;[d] and Nicolotti O.[a]

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PLATO (Polypharmacology pLATform for predictiOn) is a ligand-based polypharmacology web predictive platform designed with a two-fold main objective: a) to shortlist a number of putative protein drug targets and b) to compute the bioactivity affinity values.[1] PLATO employs a pool including 632,119 druglike ligands and 6004 targets provided with experimental annotations retrieved from the latest update of ChEMBL (release 30)[2] according to transparent filtering rules and implements two just optimized multifingerprint similarity-based algorithms, which have been recently published.[3,4]
The web framework is free accessible through a graphical user-friendly interface available online at the following link:

*http://plato.uniba.it/*

Users can interrogate PLATO by simply drawing the chemical structure of a given query or, alternatively, by pasting its SMILES notation. Two different screening options are thus given: the first is aimed at searching for putative drug targets based on molecular similarity; the second allows making quantitative predictions of bioactivity based on a statistical approach. In a few seconds, PLATO returns a standard report in a portable document format, which includes the list of the top-scored 30 predicted targets as well as a wealth of additional information for each single result regarding the ligand chemical structure, the protein drug target and the bioactivity values. The standard report also contains hyperlinks to redirect users to ChEMBL for further and deeper investigations. Please note that all the information gathered by PLATO are stored in a downloadable .json file. PLATO has been validated on thousands of external data, with performances better than those of other parallel approaches.
Overall, PLATO can be utmost importance in several applications of drug discovery: 1) for drug repurposing and further optimization studies; 2) to facilitate the identification of off-targets, thus preventing the occurrence of undesired side effects ; and 3) in combination with docking and *de novo* design studies.

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PO-105

# DISCOVERY OF GUANIDINO-CONTAINING BENZIMIDAZOLES AS NOVEL ANTIFOLATES FOR TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) is a vector-borne parasitic infection caused by Trypanosoma brucei (Tb) parasite. The drugs currently on the market are characterized by high toxicity and require long periods of treatment. Moreover, many of them have been used in therapy for more than 50 years thus causing the appearance of resistance phenomena. These conditions claim the need of addressing more adequate therapies. During evolution, protozoa belonging to the genus Trypanosoma and Leishmania have developed a biochemical supply and reuse of folate and other pteridinic products from the infected host for the biosynthesis of nucleic acids and proteins. Thus, folate enzymes, namely dihydrofolate reductase (DHFR) and pteridine reductase-1 (PTR-1) have been recognized as promising drug targets for the treatment of parasitic diseases.[1] Recently, the antimalarial drug cycloguanil (CYC) and some related dihydrotriazines have showed to be dual targeting DHFR and PTR-1 inhibitors of *T. brucei*, although with a different profile.[2] On the wave of this finding, we have recently developed a novel library of compounds, the 2-amino[1,2-a]benzimidazole derivatives (A) bearing the amino triazine moiety of CYC fused with a benzimidazole ring, and the 2-guanidinobenzimidazoles (B), as open ring analogues endowed with a greater flexibility. The compounds, tested for their on-target activity (PTR-1 and DHFR), have exhibited a preferential affinity for *Tb*DHFR, sometimes reaching nanomolar Ki values and a selectivity index more favorable for protozoan DHFR than human enzyme. Molecular docking simulations demonstrated high correlation with enzymatic inhibition assays, corroborating our hypothesis that the structure with the open guanidine group binds to both proteins with more favourable poses than that with closed ring. The conceived library has been tested in cell-based assays, proving to be non-toxic and effective against *T. brucei* in the low micromolar range. In perspective, we have identified a highly selective and potent *Tb*DHFR inhibitor of series B, worthy of further investigations in drug combination tests.

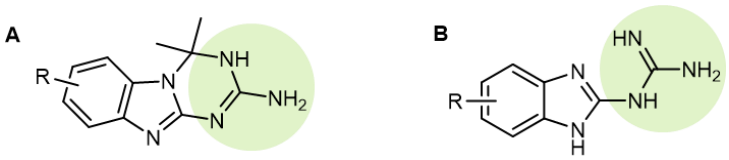


**Figure 1.** General structures of the investigated guanidino-containing benzimidazoles.

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PO-106

# DEVELOPMENT OF ENVIRONMENTALLY SUSTAINABLE CHROMMATOGRAPHIC METHODS FOR AN OVERALL ACHIRAL AND CHIRAL ANALYSIS OF AMINO ACIDS CONTAINING FOOD SUPPLEMENTS

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In this study environmentally friendly liquid chromatography methods were developed for the overall achiral and chiral analysis of an amino acid (AA) pool in a commercial food supplement.
It was clearly demonstrated that water/ethanol (EtOH) containing mobile phases allow obtaining high statistical quality methods comparable to those usually applied for the same purpose. Indeed, very appreciable performances were obtained by replacing the largely employed and toxic acetonitrile with the alcoholic component.[1]
A direct achiral ion-pairing reversed-phase HPLC (IP-RP-HPLC) method was optimized under gradient conditions to accurately quantify the eight AAs (that is, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) in the food supplement.[2,3] The data collected in this work further confirmed that very low concentrations (0.1%, by volume) of heptafluorobutyric acid can be conveniently used for the selective and efficient analysis of a heterogeneous pool of AAs under both isocratic and gradient conditions, without the need for pre-analytical derivatization steps. The method was validated for leucine and phenylalanine showing a relevant statistical quality in terms of linearity, accuracy (Recovery % in the range 98.3% - 100%), precision (RSD% in the range 1.5% - 1.9%) and limit of quantification. A concentration of 1.5 mg/mL of the selected dietary supplement was found to be optimal for quantitative analysis.
In the second part of the work a chiral chromatography method based on the use of a teicoplanin chiral stationary phase was developed with a water-EtOH (60:40, v/v) RP eluent with low amount (0.1%, by volume) of acetic acid, which allowed the use of an evaporative light scattering detector.[4] This enantioselective method has produced excellent direct enantioseparations (with $\alpha$ and $R_S$ values up to 4.6 and 15.0, respectively) of all the investigated AAs.
The methods developed revealed that (i) the content of six out of eight AAs was consistent with the manufacturer declaration (from approximately 90% up to 95%), the lower amounts of methionine (close to 80%) and tryptophan (close to 70%) were tentatively attributed to oxidative degradation; (ii) all AAs were present exclusively as L enantiomers.
Very profitably, it was demonstrated that a 2D achiral-chiral configuration is possible in the practice.[5]
Finally, a molecular modelling investigation revealed interesting insights into the enantiorecognition mechanism

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PO-107

# SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF PSEUDOPALINE AND ITS MOLECULAR SIMPLIFICATION DERIVATIVES AS CANDIDATES FOR ZINCOPHORE-ANTIBIOTICS CONJUGATES DEVELOPMENT

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Bacterial resistance against most antibiotics, represents a severe burden on public health and evolves by different mechanisms. Among them, the permeability barrier of outer-membranes is a significant factor contributing to the antibiotic resistance of Gram-negative bacteria. This suggests that the exploration of multiple strategies employed for bacterial proliferation and invasion is crucial for developing new drugs.[1] Divalent metals (Mn, Fe, Co, Ni, Cu and Zn) are essential micronutrients for all living organisms, and their uptake is vital particularly for bacterial pathogens in the context of host-pathogen interactions.[2] In metal-poor conditions, a common bacterial strategy consists in the biosynthesis of metallophores. These metal-chelating secondary metabolites sequester and import metals from the extracellular medium through dedicated membrane transporters, resulting in enhanced bacterial virulence.[3] In this context, exploiting metallophore-dependent metals uptake systems as gates in the bacterial envelope could help the development of new antibiotic therapies, such as "Trojan horse" siderophore-antibiotic conjugates.[1] In the past few years, several new metallophores have been identified and two new members of this class, staphylopine and pseudopaline, biosynthesized by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, have been widely studied. Evidences reported in literature, suggest that these compounds might play a pitoval role in the development of human pulmonary infections. Notably, the interest on pseudopaline, as potential source of new therapeutic intervention, has increased due to the huge impact of *P. aeruginosa* infections on cystic fibrosis patients.[4] Pseudopaline and staphylopine are structurally related to nicotianamine, a metal-chelator ubiquitous in higher plants, sharing an aminobutyrate moiety as structural backbone. Pseudopaline differs from staphylopine by the presence of a L-histidine moiety instead of D-histidine, and an α-ketoglutarate moiety instead of a pyruvate. Taking into account the common structural characteristics, we performed the total synthesis of pseudopaline and two molecular simplification derivatives, in order to conduct a structure-activity relationships study and to identify the best candidate for the development of a zincophore-antibiotic conjugate. In particular, for simplified compounds, by keeping fixed the aminobutyrate portion and the stereochemistry, we removed the histidine residue or both histidine and α-ketoglutarate fragments. The effect of pseduopaline and its analogs on *P. aeruginosa* mutant strains growth will be discussed in detail.

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PO-108

# PROTECTIVE EFFECT OF COCOA IN A MODEL OF PARKINSON'S DISEASE VIA ENDOPLASMIC RETICULUM STRESS INHIBITION

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The endoplasmic reticulum (ER) plays a pivotal role for $Ca^{2+}$ homeostasis, controlling synthesis, and protein folding. During cellular stress, variations in ER homeostasis and its functioning occurs due to accumulated misfolded or unfolded proteins in the ER. This condition is referred as ER stress. Therefore, a cascade of signaling events termed unfolded protein response (UPR) is activated as adaptative response to alleviate the ER stress condition.[1,2] Activating transcription factor 6 (ATF6), protein kinase R-like ER kinase (PERK), and inositol-requiring enzyme 1 (IRE1) represent the three main transmembrane proteins initiating UPR signaling in ER stress response. In normal conditions they are maintained in an inactive state. When ER stress is triggered, they are activated through dissociation from GRP78/binding protein (BIP), the master regulator chaperone of UPR, which aids proper folding of misfolded proteins.[3] A lot of evidence suggests that natural products target the ER stress signaling pathway, exerting a potential action in neurodegenerative diseases like Alzheimer's and Parkinson's.[4,5] Polyphenols-rich cocoa has been shown many benefits on human health including antioxidative and anti-inflammatory effects. The antioxidant action of cocoa is due to its high content in flavonoids, such as epicatechin, catechin, and proanthocyanidins, naturally occurring in this matrix.[6] The goal of our study was to investigate whether the known antioxidant properties of cocoa have any influence on Parkinson's disease through modulation of unfolded protein response (UPR) examining molecular mechanisms of endoplasmic reticulum stress activated-pathways. Our findings demonstrate the ability of cocoa to specifically targets PERK sensor, with significant antioxidant and antiapoptotic activities as crude and fractioning extract in 6-hydroxydopamine induced SH-SY5Y cell model of Parkinson's disease.

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PO-109

# NOVEL WATER-SOLUBLE NAPHTHALIMIDE-POLYAMINE CONJUGATES FOR ANTICANCER PHOTODYNAMIC THERAPY AND DIAGNOSIS

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Photodynamic therapy (PDT) is a promising therapeutic modality based on the combined use of light and photosensitizers, approved for treating several types of cancers.[1] Under light irradiation, thio-heterocyclic naphthalimide-based photosensitizers showed both antitumor effects by generating reactive oxygen species (ROS), and favorable fluorescent properties.[2] Thus, naphthalimides emerged as valuable anticancer theranostic agents that can provide, at the same time, diagnosis (via fluorescence imaging) and treatment (via ROS production) with high spatiotemporal precision.[2] However, no effective conjugation strategy for simultaneously endowing naphthalimides with water solubility and efficient ROS production ability currently exists. Herein, novel water-soluble naphthalimide-polyamine conjugates **1**-**4** were developed by incorporating natural and synthetic polyamines to a naphthalimide scaffold (Figure 1). The incorporation of polyamine chains, which differ in chain length and number of nitrogen atoms, allowed to obtain naphthalimide conjugates with: (i) good photophysical, photochemical, and fluorescent properties, (ii) a water-soluble profile, (iii) improved anti-cancer photodynamic activity in MCF-7 breast cancer cells, and (iv) negligible dark toxicity.

Overall, this novel class of naphthalimide-polyamine conjugates provides a promising conjugation strategy for improving drug-likeness and enhancing photodynamic therapeutic efficiency of naphthalimide-based photosensitizers.

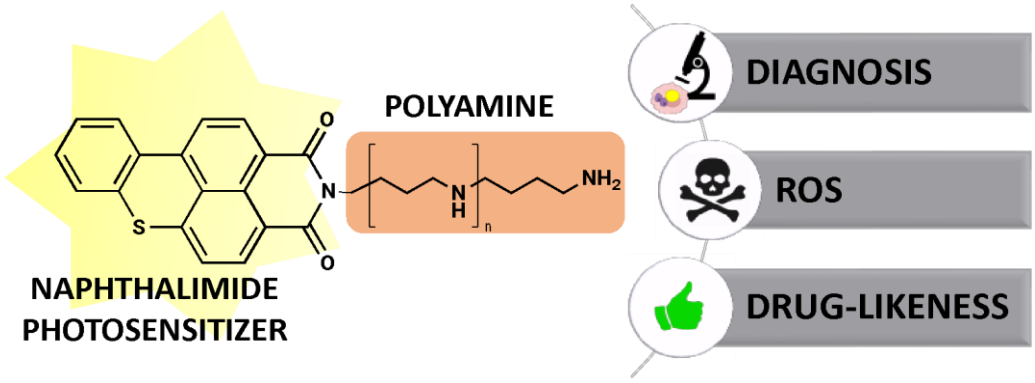


**Figure 1.** Conjugation strategy of naphthalimide-polyamine conjugates.

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PO-110

# SYNTHESIS AND BIOLOGICAL EVALUATION OF SUCROSE–BASED GLYCOLIPID SURFACTANTS AS ANTIMICROBIAL AND PERMEABILITY ENHANCER AGENTS

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A large number of antimicrobial agents present several limitations due to toxicity, spectrum of activity, safety and cross-resistance. Consequently, the research for new drugs is still fundamental. In this context, sugar-based esters surfactants represent interesting opportunity due to their non-toxic, biocompatible, and biodegradable profile.[1,2] Moreover, they exhibit relevant permeability enhancer properties.[3,4] Among sugar-based surfactants, saturated sucrose esters (SEs) are the most studied derivatives,[5] while SEs with unsaturated fatty acid or aryl(alkyl) chains have received less attention.

In this study, a small library of SEs glycolipids (Figure 1) has been synthesized and tested to determine the minimum inhibitory concentration (MIC) values against different bacteria and fungi. Furthermore, cytotoxicity and permeability studies were conducted in comparison to analogue lactose aromatic esters.

An appropriately modified Mitsunobu procedure[6] was applied to the synthesis of SEs. The reaction was more versatile for different acids (in particular aromatic and unsaturated) in comparison to the enzymatic and classical esterifications. In detail, the reaction was regioselective for the position 6 and permitted to obtain the desired products in good yields with a reduction of undesired derivatives (e.g., diesters). The antibacterial activity of SEs was mainly observed against Gram-positive bacteria. Remarkably, unsaturated SEs resulted the most active against all the selected fungi. Best result was achieved with sucrose palmitoleate (C16:1) against *C. albicans* strain. This latter derivative was selected for further studies and compared with its precursors sucrose and palmitoleic acid. About aryl(alkyl) SEs permeability studies, a desired reversible effect on the trans-epithelial electrical resistance (TEER) was observed. Additionally, sucrose *p*-phenylbenzoate improved the fluorescin isothiocyanate FITC-dextran permeability across Calu-3 better than the control and its lactose analogue.

In conclusion, unsaturated SEs could represent suitable antifungal agents while aryl(alkyl) SEs demonstrated to be good permeability enhancers for pharmaceuticals applications.

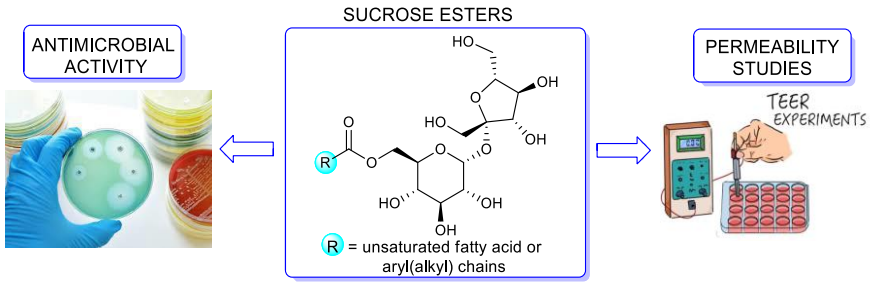


**Figure 1.** Schematic representation of sucrose esters and relative biological studies.

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# NATURAL COMPOUNDS TARGETING *E. COLI* WRBA AS ANTIBIOFILM AGENTS: VIRTUAL SCREENING, DESIGN AND SYNTHESIS OF ANALOGS, BIOLOGICAL EVALUATION

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Resistance to antibiotics has become one of the main concerns of modern medicine. This phenomenon is worsened by biofilm formation, which frequently leads to treatment failures.[1] Hence, anti-biofilm agents represent useful tools in the treatment of microbial infections, increasing the effectiveness of antibiotics and preventing resistance mechanisms.

Different studies have demonstrated that the protein WrbA plays a key role in the formation of biofilm in *E. coli*. Nonetheless, its specific function is still poorly understood, and only a few inhibitors are reported in the literature.[2] For this reason, our research group has worked towards the identification of new WrbA inhibitors as antibiofilm agents.

Through a target-based virtual database screening, we identified natural products endowed with a high affinity towards WrbA; the most promising compounds were purchased for biophysical and biological evaluation.

Hence, we selected a natural scaffold as a starting point to create a new library of organic molecules and perform a virtual screening. Finally, five compounds were selected to be synthesized and subjected to biophysical evaluation.

Both the natural and synthetic compounds were tested on recombinant *E. coli* WrbA by MicroScale Thermophoresis (MST), which allowed the determination of their dissociation constants ($K_d$). Among the natural compounds, one of them displayed a $K_d$ in the nanomolar range. This value was lower than those measured for both caffeic and zosteric acid, which are known WrbA inhibitors.[3] On the other hand, the synthesized compounds bound WrbA with $K_d$ values in the low micromolar range. Based on our encouraging results, the inhibitors were assayed on *E. coli* and *S. aureus* to verify their antibiofilm effect. The outcomes of our study will be presented.

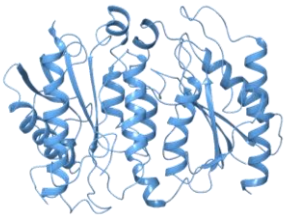

**Figure 1.** *E. coli* WrbA structure represented as a ribbon.

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PO-112

# NEW STRATEGIES TO OVERCOME BIOFILM FORMATION BY NATURAL PRODUCT DERIVATIVES

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Biofilm is a microbial community in which microorganisms are tightly bound to each other and enclosed in a self-produced matrix (EPS). Bacterial biofilm is frequently present on moisty surfaces, food, teeth, medical devices, water systems etc. In humans, biofilms can be damaging for the health, causing and sustaining chronic infections due to their high antibiotic resistance, but also beneficial (*e.g.*, microbiome).
Among the different approaches to prevent biofilm formation, the new trend is to consider antibiofilm agents acting at sublethal concentrations. In this way, the compounds inhibit biofilm formation without killing the bacteria, thus avoiding resistance selection. Based on our previous experience in the use of LDPE, covalently functionalized with natural product derivatives (*p*-aminosalicylic and *p*-aminocinnamic acids), for the development of antifouling materials for medical devices[1,2], we decided to improve and adapt our validated strategy to silica nanoparticles. Therefore, we functionalized the starting material (Ludox HS-40) with the above-mentioned natural derivatives, using linkers differing in nature and length, to obtain nanoparticles (Figure 1) suitable for the coating of various surfaces. The obtainment of the new systems, their characterization, the preparation of the coating, and the biological evaluation of their antibiofilm activities will be presented.

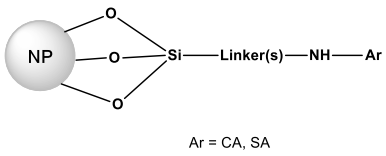


**Figure 1**. Schematic representation of the new Ludox HS-40 derivatives.

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PO-113

# DIPROPYLEN GLYCOL DIMETHYL ETHER (DME), NEW GREEN SOLVENT FOR SOLID PHASE PEPTIDED SYNTHESIS: FURTHER CHALLENGES TO IMPROVE SUSTAINABILITY IN THE DEVELOPMENT OF THERAPEUTIC PEPTIDES

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Peptides play an important role in the fields of drug discovery. Solid-phase peptide synthesis (SPPS) is generally the method of choice for the chemical synthesis of peptides. Today, synthetic strategies are moving towards increasingly eco-sustainable approaches. However, the individual steps of the SPPS cannot be considered green due to the low atom economy, a large amount of waste generated, the environmental impact of solvents and reagents. It is, therefore, necessary to make the synthesis of peptides greener. The focus is on the search for a solvent that reflects the parameters of green chemistry, and at the same time, is applicable to all phases of peptide synthesis. Dipropylen glycol dimethyl ether (DME) is a solvent that does not show any toxicity and is safe for the environment and for humans. This solvent, in fact, according to Regulation (EC) N. 1272/2008, was classified as a non-substance dangerous. Furthermore, the chemical-physical properties are comparable to the solvent most used in SPPS, N, N-dimethylformamide (DMF), such as viscosity, the boiling point and flash point. These characteristics make it an excellent green solvent.
However, some tests needed to evaluate its applicability to all the steps of the SPPS. The ability of the solvent to solubilize commonly employed protected amino acids and coupling reagents was investigated. Furthermore, another important step for SPPS is the choice of the resin and its swelling degree. A good solvent, therefore, must be able to swell different resins on the market. In this regard, the swelling test was carried out with DME in six different resins. Finally, DME was tested throughout the deprotection phase of the Fluorenylmethyloxycarbonyl (Fmoc) group. During the tests the green solvent showed excellent properties. Further investigations will be carried out to make it scalable on an industrial level.

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# ADVANCES TOWARD THE DISCOVERY OF NOVEL 3-BENZYLQUINOLIN-2(1*H*)-ONES AS POTENT AGONISTS OF THE GPR55 RECEPTOR

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**Introduction:** GPR55 is a G protein-coupled receptor initially considered as an orphan receptor and then proposed as the "type 3" cannabinoid receptor, even if its categorization is still being studied. Recently, it has emerged as an innovative therapeutic target for the treatment of neurodegenerative diseases due to its highest expression in microglia cells and its involvement in the regulation of neuroinflammatory processes. However, the molecular mechanisms underlying this action have not been yet completely understood. Recent evidences highlighted neuroprotective effects exerted by GPR55 agonists in neural stem cells.[1] Another recent study suggested an anti-neuroinflammatory effect produced by GPR55 antagonists/inverse agonists[2] in LPS-activated primary microglial cells.[3] In this context, the development of novel and potent GPR55 modulators is extremely important to better understand the peculiar role of GPR55 in neuroinflammation.
Starting from 3-benzylcoumarin derivatives recently reported as GPR55 antagonists/inverse agonists, we developed a novel series of 3-benzylquinolin-2(1*H*)-ones (**A**, *figure 1*), which resulted to be among the most potent GPR55 agonists synthesized to date. Moreover, some of these compounds showed complete selectivity over one or both CBRs. Thus, we expanded the set in order to deepen the preliminary outlined structure-activity relationships for this class of molecules, by increasing or reducing the length of the *n*-butyl chain at position R7 or by introducing an hydrogen atom at position R11 (**B**, *figure 1*). Some of the newly synthesized compounds displayed affinity ($K_i$) values included in the low nanomolar range, maintaining agonist activity at GPR55 receptor or showing some degree of inverse agonism.

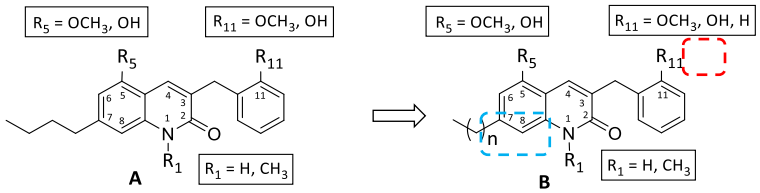


**Figure 1.** Structural modification leading to the extension of the 3-benzylquinolin-2(1*H*)-one series.

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