Università degli Studi
"G. d'Annunzio"
Chieti - Pescara

Dipartimento di Farmacia

XXVIII
National Meeting on
Medicinal Chemistry

7N 15P 6C 9F 15

15th Young Medicinal Chemists' Symposium
Nuove Prospettive in Chimica Farmaceutica

17th-20th September 2023
Chieti, University "G. d'Annunzio" of Chieti-Pescara

Società Chimica Italiana
Divisione di Chimica Farmaceutica

BOOK OF ABSTRACTS

WELCOME message

On behalf of the Scientific and Organizing Committees, it is our great pleasure to invite you to the 28th National Meeting on Medicinal Chemistry (NMMC28), the flagship event of the Divisione di Chimica Farmaceutica (Division of Medicinal Chemistry) of the Società Chimica Italiana (Italian Chemical Society).

The 2023 NMMC edition will be organized by the University "G.d'Annunzio" of Chieti-Pescara in cooperation with local authorities and major national and international pharmaceutical companies.

NMMC28, together with the 15th Young Medicinal Chemists Symposium "Nuove Prospettive in Chimica Farmaceutica" (NPCF15), aims to promote scientific research and coordinate collaborations by bringing together experts from the academic and industrial medicinal chemistry sector from all over Italy and beyond.

The meeting will cover advances in medicinal chemistry and drug discovery in major therapeutic areas, including infectious, neurodegenerative and rare diseases, and cancer. In parallel scientific sessions, the most recent advances in computer-aided drugs design and bioanalytical techniques applied to drug discovery, in sustainable medicinal chemistry, bioactive peptides and nutraceuticals will be also featured. More importantly, participants will have the chance to present their work, establish and strengthen collaborative networks, a key for successful research.

The meeting will start on September 17th with the Opening Ceremony at the Rectorate Auditorium of the University "G. d'Annunzio" of Chieti-Pescara (Chieti Campus), and will continue at the Aula magna "Giancarlo Bettoni" (Department of Pharmacy) from 18th to 20th September. It will also be the opportunity to greet the winners of the DCF prizes, at the Opening Ceremony. The scientific contributions will be divided into two parallel sessions, with the participation of distinguished international scientists and will host

- 5 plenary lectures by invited international key opinion leaders
- 1 Pratesi Medal Lecture
- 10 keynote lectures on pioneering themes
- 34 oral communications
- 20 Flash oral Communication
- Two poster sessions

More than 300 colleagues from academia and industry are expected. Upon application, several grants for young scientists, covering the full registration fee and accommodation, will be provided by DCF-SCI and other Institutional Sponsors.

We are looking forward to welcoming you all in Chieti!

Prof. Maria Laura Bolognesi
DCF-SCI, President

Prof. Adriano Mollica
Local Organizing Committee, Chair

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- Maria Laura Bolognesi (Chair, University of Bologna, IT)
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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-ISCB 2023
EFMC International Symposium on Chemical Biology
Basel, Switzerland | November 16-18, 2023

EFMC-ACSMEDI Medicinal Chemistry Frontiers 2024
Joint Symposium on Medicinal Chemistry
Utrecht, The Netherlands | April 8-11, 2024

18th EFMC Short Course on Medicinal Chemistry
Oegstgeest, The Netherlands | April 21-24, 2024

EFMC-ISMC 2024
XXVIII EFMC International Symposium on Medicinal Chemistry
Rome, Italy | September 1-5, 2024

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
— The "EFMC-WuXi AppTec Award for Excellence in Chemical Biology"
Visit www.efmc.info/awards for more information

Prizes

— EFMC Prizes for Young Medicinal Chemists in Industry & Academia
— EFMC-YSN PhD Prize
— Symeres PhD Prize for Excellence in Chemistry in Life Sciences Research
Visit www.efmc.info/prizes for more information

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, 17th September

14.30	**REGISTRATION**
	Auditorium Rectorate (University "G. d'Annunzio" of Chieti-Pescara)
17.00	**OPENING CEREMONY** **Liborio Stuppia,** Rector of the University "G. d'Annunzio" of Chieti-Pescara **Amelia Cataldi,** Head of the Department of Pharmacy of the University "G. d'Annunzio" of Chieti-Pescara **Luigi Brunetti,** President of the Faculty of Pharmacy of the University "G. d'Annunzio" of Chieti-Pescara **Maria Laura Bolognesi,** President of the Medicinal Chemistry Division - Italian Chemical Society **Adriano Mollica,** Chair of the Local Organizing Committee
17.30	**Chair: Maria Laura Bolognesi,** President of the Medicinal Chemistry Division - Italian Chemical Society **MEDICINAL CHEMISTRY DIVISION - ITALIAN CHEMICAL SOCIETY AWARDS** **Chiara Borsari,** University of Milan, Italy **Antonella Ciancetta,** University of Ferrara, Italy **BEST DOCTORAL THESIS AWARDS** **FC: Francesca Annunziata,** University of Milan, Italy **DEVELOPMENT OF SUSTAINABLE AND EFFICIENT TAILOR-MADE PROCESSES FOR PHARMA AND FOOD APPLICATIONS** **FC: Giulia Bononi,** University of Pisa, Italy **DEVELOPMENT OF THERAPEUTIC AND DIAGNOSTIC AGENTS TARGETING TUMOR LIPID AND SUGAR METABOLISM**
18.00	**PRATESI MEDAL, MEDICINAL CHEMISTRY DIVISION - ITALIAN CHEMICAL SOCIETY** **Dario Neri,** Philogen S.p.A., Italy & ETH Zürich, Switzerland **THE DREAM OF PAUL EHRLICH**
18.45	**PL1: Kelly Chibale,** University of Cape Town, South Africa **MEDICINAL CHEMISTRY APPROACHES TO REPOSITIONING ANTICANCER HUMAN KINASE INHIBITORS FOR MALARIA**
20.00	**WELCOME BUFFET**

Monday, 18th September

	Aula Magna (Department of Pharmacy)	Aula 3 (Department of Pharmacy)
	Chair: Adriano Mollica	
9.00	**PL2: Steven Ballet,** Vrije Universiteit Brussel, Belgium **DEVELOPMENT OF GENERIC G PROTEIN PEPTIDOMIMETICS ABLE TO STABILIZE ACTIVE STATE G_s PROTEIN-COUPLED RECEPTORS FOR APPLICATION IN DRUG DISCOVERY**	
	Chairs: Adriano Mollica, Francesco Epifano	**Chairs: Patrizia Diana, Salvatore Genovese**
10.00	**KN1: Paolo Rovero,** University of Florence, Italy **THE CONTRIBUTION OF PEPTIDE RESEARCH TO THE FIGHT AGAINST SARS-COV-2: FROM VACCINES TO ANTIVIRALS**	**KN2: Alessia Bertamino,** University of Salerno, Italy **DESIGN AND DEVELOPMENT OF NEW SOLUBLE EPOXIDE HYDROLASE INHIBITORS AS PHARMACOLOGICAL TOOLS IN INFLAMMATORY DISEASE**
10.30	**OC1: Marina Sala,** University of Salerno, Italy **GREENING THE SOLID-PHASE PEPTIDE SYNTHESIS: FURTHER CHALLENGES TO IMPROVE SUSTAINABILITY IN THE DEVELOPMENT OF THERAPEUTIC PEPTIDES**	**OC2: Valeria Pittalà,** University of Catania, Italy **RECENT ADVANCES IN THE DISCOVERY OF HEME OXYGENASE-1 INHIBITORS WITH ANTICANCER ACTIVITY**
10.50	**FC1: Michela Buonocore,** University of Salerno, Italy **EXPLOITING THE FEATURES OF SHORT PEPTIDES TO RECOGNIZE SPECIFIC CELL SURFACE MARKERS**	**FC2: Manuela Giovanna Basilicata,** University of Salerno, Italy **IN VITRO CHEMICAL AND METABOLIC STABILITY AND IN VIVO PHARMACOKINETIC STUDIES OF A CONFORMATIONALLY RESTRICTED RETIGABINE ANALOGUES AS NOVEL NEURONAL KV7 CHANNEL ACTIVATORS**
11.00	**COFFEE BREAK**	
11.20	**OC3: Federica Moraca,** University of Naples "Federico II", Italy **DISCOVERING ALZHEIMER'S A β-AMYLOID FIBRILLOGENESIS INHIBITOR PEPTIDES THROUGH A COMBINED 3D-GRID PHARMACOPHORE AND METADYNAMICS STRATEGY**	**OC6: Alessandra Anna Altomare,** University of Milan, Italy **THINNED APPLE POLYPHENOLS ALLEVIATE INFLAMMATION IN A MOUSE MODEL OF DNBS-INDUCED COLITIS: LABEL-FREE QUANTITATIVE PROTEOMICS STUDIES**
11.40	**OC4: Emanuela Salviati,** University of Salerno, Italy **FLEXIBLE SPATIAL-MASS SPECTROMETRY STRATEGY APPLIED TO TRAUMATIC BRAIN INJURY AND ITS ASSOCIATION WITH ALZHEIMER'S DISEASE**	**OC7: Marco Paolino,** University of Siena, Italy **DONEPEZIL-LIKE DUAL TARGET AChE AND MAO-B INHIBITORS WITH PHOTOMODULABLE INHIBITORY ACTIVITY**

12.00	**OC5: Claudia Mugnaini,** University of Siena, Italy **A DUAL-TARGET APPROACH FOR THE TREATMENT OF ALZHEIMER'S DISEASE**	**OC8: Filippo Basagni,** University of Bologna, Italy **FYN/GSK-3β DOUBLE KINASE INHIBITORS TO INVESTIGATE NEUROINFLAMMATORY PROCESSES IN NEURODEGENERATIVE DISEASES**
12.30 **14.00**	**LUNCH** **POSTER SESSION & COMMERCIAL EXHIBITION**	
	Aula Magna (Department of Pharmacy)	
	Chair: Stefano Alcaro	
15.10	**PL3: Zoe Cournia,** Biomedical Research Foundation, Academy of Athens, Greece **EXPLOITING ALLOSTERY FOR COMPUTER-AIDED DRUG DESIGN TO TARGET ONCOGENES**	
	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Stefano Alcaro, Violetta Cecchetti	**Chairs: Gianluca Sbardella, Stefano Moro**
16.00	**KN3: Alessandro Accetta,** Chiesi Farmaceutici, Italy **MEDICINAL CHEMISTRY OF INHALED DRUGS: EXAMPLES FROM VALIDATION TO PRECLINICAL CANDIDATES**	**KN4: Giovanni Bottegoni,** University of Urbino, Italy ***IN SILICO* POLYPHARMACOLOGY STUDIES FOR CNS CONDITIONS**
16.30	**OC9: Bruno Cerra,** University of Perugia, Italy **INTEGRATED FLOW PLATFORM TO STREAMLINE THE SYNTHESIS OF BIOLOGICALLY-ACTIVE STEROIDS**	**OC11: Valentina Straniero,** University of Milan, Italy **PK7: A SYNAPTIC TARGETING COMPOUND TO OVERCOME PARKINSON'S DISEASE**
16.50	**OC10: Vincenzo Maria D'Amore,** University of Naples "Federico II", Italy **A DYNAMICAL AND ATOMISTIC RESOLUTION VIEW OF THE G PROTEIN COUPLED RECEPTOR A2A ACTIVATION MECHANISM**	**OC12: Federico Ricci,** University of Messina, Italy **LEARNING ON HUMAN TYROSINASE: FROM HOMOLOGY MODELLING TO NEW INHIBITORS SELECTION**
17.10	**FC3: Elisa Patacchini,** "Sapienza" University of Rome, Italy **STRUCTURE-BASED DRUG DESIGN OF NEW SIGMA1 AGONISTS FOR HUNTINGTON'S DISEASE TREATMENT**	**FC4: Alessandro Andreani,** IIT, Genova, Italy **TARGETING THE CONSERVED ACTIVE SITE OF SPLICING MACHINES WITH SPECIFIC AND SELECTIVE SMALL MOLECULE MODULATORS**
17.30	**COCKTAIL AND JAZZ CONCERT at Teatro Marrucino, Chieti**	

Scientific Program / Tuesday, 19th September

	Aula Magna (Department of Pharmacy)	Aula 3 (Department of Pharmacy)
	Chair: Gianluca Sbardella	
9.00	**PL4: Thomas Lundbäck,** AstraZeneca, Gothenburg, Sweden **APPLICATION OF THE CELLULAR THERMAL SHIFT ASSAY IN DRUG DISCOVERY**	
	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Gianluca Sbardella, Marco De Vivo	**Chairs: Giancarlo Aldini, Cristina Maccallini**
10.00	**KN5: Simona Collina,** University of Pavia, Italy **SMALL MOLECULES INTERFERING WITH HUR–RNA COMPLEXES: A PROMISING STRATEGY TO DISCOVER ANTICANCER AGENTS**	**KN6: Enrica Calleri,** University of Pavia, Italy **APPLICATIONS OF PROTEIN-BASED ANALYTICAL METHODS IN DRUG DISCOVERY**
10.30	**OC13: Marta Serafini,** University of Oxford, United Kingdom **THE DEVELOPMENT OF HYPOXIA-ACTIVATED PROTACS (HAP-TACS) TO SELECTIVELY DEGRADE BRD4 IN HYPOXIC CANCER CELLS**	**OC14: Lorenza Marinaccio,** University "G. d'Annunzio" of Chieti-Pescara, Italy **LYCOPENE EXTRACTION WITH α-PINENE, NATURAL VOLATILE DEEP EUTECTIC SOLVENT MENTHOL-THYMOL AND EXTRA VIRGIN OLIVE OIL**
10.50	**FC5: Camilla Pecoraro,** University of Palermo, Italy **DISCOVERY OF PYRAZOLE-PYRIMIDINES AS NOVEL CDK7 INIHIBITORS: SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION**	**FC6: Chiara Lambona,** "Sapienza" University of Rome, Italy **TARGETING ROS PRODUCTION THROUGH INHIBITION OF NADPH OXIDASE**
11.00	**COFFEE BREAK**	
	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Ivana Cacciatore, Cosimo D. Altomare	**Chairs: Giancarlo Aldini, Letizia Giampietro**
11.20	**OC15: Marco Lolli,** University of Torino, Italy **POTENT HUMAN DIHYDROOROTATE DEHYDROGENASE INHIBITORS BASED ON THE 2-HYDROXYPYRAZOLO [1,5-A]PYRIDINE SCAFFOLD AGAINST ACUTE MYELOGENOUS LEUKEMIA: BE PREPARED TO THE CLINIC**	**OC18: Serena Montanari,** University of Bologna, Italy **MICROALGAE: BIOACTIVES EXTRACTION AND CHARACTERIZATION FOR NUTRACEUTICAL USAGE**
11.40	**OC16: Luca Pinzi,** University of Modena, Italy **DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF HSP90/HDAC6 DUAL INHIBITORS BASED ON A 2-AMINO-PYRROLOPYRIMIDINE SCAFFOLD FOR THE TARGETING OF AGGRESSIVE PROSTATE CANCER**	**OC19: Erika Baldini,** University of Ferrara, Italy **CHEMICAL CHARACTERIZATION AND VALORIZATION OF *Helichrysum italicum* AND MEDICINAL MUSHROOMS FOR NUTRACEUTICAL APPLICATIONS**

12.00	**OC17: Anna di Porzio,** University of Naples "Federico II", Italy **DEVELOPMENT OF A NOVEL MULTI-TARGET ANTICANCER APPROACH BASED ON G-QUADRUPLEX STABILIZATION AND INHIBITION OF HUMAN CARBONIC ANHYDRASES**	**OC20: Greta Petrella,** University of Rome "Tor Vergata", Italy **A PERSONALIZED URINARY METABOLIC PROFILE FOR BLADDER CANCER PATIENTS: AN APPLICATION OF THE SYNHMET METHOD**
12.30 **14.00**	**LUNCH** **POSTER SESSION & COMMERCIAL EXHIBITION**	
	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Isabella Romeo, Chiara Borsari	**Chairs: Azzurra Stefanucci, Antonella Ciancetta**
15.30	**KN7: Chiara Borsari,** University of Milan, Italy (Premio DCF) **MEDCHEM STRATEGIES TO SELECTIVELY TARGET THE PI3K-mTOR PATHWAY FOR ONCOLOGY APPLICATIONS**	**KN8: Antonella Ciancetta,** University of Ferrara, Italy (Premio DCF) **MIDAS: A COMBINED ENHANCED SAMPLING MOLECULAR DYNAMICS AND FRAGMENT-BASED APPROACH TO LOCATE ALLOSTERIC BINDING SITES IN G PROTEIN-COUPLED RECEPTORS**
16.00	**FC7: Michela Puxeddu,** "Sapienza" University of Rome, Italy **NOVEL PYRROLE TUBULIN ASSEMBLY INHIBITOR AS ANTICANCER AGENT INDUCING FERROPTOSIS**	**FC13: Anna Marta Pasieka,** University of Bologna, Italy **APPLICATION OF CLICK CHEMISTRY IN THE DEVELOPMENT OF NOVEL ANTILEISHMANIAL PROTEOLYSIS TARGETING CHIMERAS**
16.10	**FC8: Jessica Sebastiani,** "Sapienza" University of Rome, Italy **RS6077 AS NEW INDUCER OF CELL DEATH IN A LYMPHOMA TUMOR IN VIVO**	**FC14: Matteo Lusardi,** University of Genova, Italy **STRUCTURAL FUNCTIONALIZATION OF TRI-SUBSTITUTED PYRAZOLE DERIVATIVES AND PRELIMINARY EVALUATION OF THEIR ANTIMALARIAL ACTIVITY**
16.20	**FC9: Rosa Sparaco,** University of Naples "Federico II", Italy **MOLECULAR HYBRIDS BETWEEN ANTIGLAUCOMA DRUGS AND H2S DONORS: SYNTHESIS AND IN VITRO H2S RELEASING PROPERTIES**	**FC15: Francesco Melfi,** University "G. d'Annunzio" of Chieti-Pescara **NATURE-INSPIRED ANTIBACTERIAL AGENTS: SYNTHESIS AND BIOLOGICAL ACTIVITY OF EUGENOL DERIVATIVES AGAINST H. pylori STRAINS**
16.30	**FC10: Francesco Samarelli,** University of Bari, Italy **NOVEL AZEPINO[4,3-b]INDOLE DERIVATIVES AS LIGANDS OF THE CANNABINOID-ACTIVATED ORPHAN RECEPTORS GPR18 AND GPR55 WITH POTENTIAL AGAINST NEURODEGENERATIVE DISEASES**	**FC16: Letizia Crocetti,** University of Florence, Italy **EBSELEN ANALOGUES AS HUMAN NEUTHOPHIL ELASTASE (HNE) INHIBITORS AND ANTIOXIDANT AGENTS**

16.40	**FC11: Alessia Alberico,** University of Naples "Federico II", Italy **GREEN AND AFFORDABLE KETOAMIDES SYNTHESIS AS VERY POTENT BROAD-SPECTRUM COVs MAIN PROTEASE INHIBITORS: X-RAY STRUCTURE AND BIOLOGICAL PROFILE**	**FC17: Aleksei Smirnov,** University of Camerino, Italy **DUAL ANTA-INHIBITORS TARGETING PROTEIN KINASE CK1Δ AND A2A ADENOSINE RECEPTOR USEFUL IN NEURODEGENERATIVE DISORDERS**
16.50	**FC12: Giovanni Graziano,** University of Bari, Italy **N-ADAMANTYL-ANTHRANIL AMIDE DERIVATIVES: NEW SELECTIVE LIGANDS FOR THE CANNABINOID RECEPTOR SUBTYPE 2 (CB2R)**	**FC18: Emanuele Fabbrizi,** "Sapienza" University of Rome, Italy **FIRST-IN CLASS SPECIFIC INHIBITORS OF THE MITOCHONDRIAL DEACYLASE SIRTUIN 4**
17.00	**COFFEE BREAK**	
	Aula Magna (Department of Pharmacy)	
17.30	**ASSEMBLEA DELLA DIVISIONE DI CHIMICA FARMACEUTICA - SOCIETÀ CHIMICA ITALIANA (DCF-SCI GENERAL MEETING)**	
20.30	**SOCIAL DINNER at "Tenuta Di Sipio" Winery**	

Wednesday, 20th September

	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Alessandra Ammazzalorso, Mariangela Agamennone	**Chairs: Simone Carradori, Francesco Epifano**
9.00	**KN9: Enza Lacivita,** University of Bari, Italy **TARGETING FORMYL PEPTIDE RECEPTORS TO TACKLE INFLAMMATION IN NEUROLOGICAL DISORDERS AND CANCER**	**KN10: Alessia Petrocchi,** IRBM, Italy **THE DISCOVERY OF I-0436650, A POTENT AND SPECIFIC ALLOSTERIC SHP2 INHIBITOR FOR THE CANCER THERAPY**
9.30	**OC21: Samuele Maramai,** University of Siena, Italy **1,4,5-TRISUBSTITUTED 1,2,3-TRIAZOLE DERIVATIVES AS HSP90 INHIBITORS: SYNTHESIS, EVALUATION, AND STRUCTURAL CHARACTERIZATION**	**OC26: Tommaso Felicetti,** University of Perugia, Italy **FROM 2,1-BENZOTHIAZINE 2,2-DIOXIDE DERIVATIVES TO SULFONYL ANTHRANILIC ACID ANALOGUES AS POTENT PAN SEROTYPE DENGUE INHIBITORS**
9.50	**OC22: Maria Giulia Nizi,** University of Perugia, Italy **PROGRESS ON POTENT AND SPECIFIC PARP INHIBITORS: A SAR STUDY AROUND THE [1,2,4]TRIAZOLO [3,4-b]BENZOTHIAZOLE SCAFFOLD**	**OC27: Francesca Galvani,** Univeristy of Parma, Italy **QM/MM MODELLING OF MGL CARBAMOYLATION BY PIPERAZINE AZOLE UREAS REVEALS THE ROLE OF LEAVING GROUP EXPULSION AND DISCRIMINATES INHIBITORS WITH HIGH AND LOW POTENCY**

10.10	**OC23: Lidia Ciccone,** University of Pisa, Italy **MOLECULAR INTERACTION OF PCSK9 WITH AN INHIBITORY NANOBODY, CAP1 AND HLA-C:FUNCTIONAL REGULATION OF LDLR LEVELS**	**OC28: Laura Braconi,** University of Florence, Italy **TETRAZOLE AND OXADIAZOLE DERIVATIVES OF TARIQUIDAR AS POTENT MDR REVERSERS**
10.30	**OC24: Antonella Messore,** Univeristy of Rome "Sapienza", Italy **INDOLYL DIKETO ACID DERIVATIVES AS INHIBITORS OF NSP13 OF SARS-COV-2 THAT BLOCK VIRAL REPLICATION**	**OC29: Carmen Cerchia,** University of Naples "Federico II", Italy **A NEW ANTIDIABETIC AGENT SHOWING PPAR α/γ DUAL AGONISM AND MITOCHONDRIAL PYRUVATE CARRIER INHIBITION**
10.50	**OC25: Erika Del Grosso,** University of Eastern Piedmont, Italy **VALIDATION OF A NEW LC-HRMS METHOD FOR THE QUANTIFICATION OF THE CHEMICAL CHAPERONE 4-PHENYLBUTYRIC ACID (4-PBA) IN CELL CULTURE MEDIA**	**OC30: Serena Vittorio,** University of Milan, Italy **ENSEMBLE OF STRUCTURE AND LIGAND-BASED CLASSIFICATION MODELS FOR hERG LIABILITY PROFILING**
11.10	**COFFEE BREAK**	
	Aula Magna (Department of Pharmacy)	**Aula 3 (Department of Pharmacy)**
	Chairs: Cristina Campestre, Barbara De Filippis	**Chairs: Marialuigia Fantacuzzi, Serena Fiorito**
11.30	**OC31: Emanuele Amata,** University of Catania, Italy **SYNTHESIS, COMPUTATIONAL INSIGHTS AND EVALUATION OF NOVEL SIGMA RECEPTORS LIGANDS**	**OC33: Ana Caballero,** Pharmacelera, Barcelona, Spain **THE ROLE OF HYDROPHOBICITY IN DRUG DISCOVERY: FROM 3D-QSAR MODELS TO HUGE CHEMICAL LIBRARIES EXPLORATION**
11.50	**OC32: Barbara Vergani,** Italfarmaco, Milan, Italy **2-DIFLUOROMETHYL-1,3,4-OXADIAZOLE – TOWARDS ABSOLUTE SELECTIVITY VS HDAC6**	**OC34: Daniele Aiello,** University of Modena and Reggio Emilia, Italy **LEAD OPTIMIZATION OF HUMAN THYMIDYLATE SYNTHASE DIMER DISRUPTERS: FROM COMPUTATIONAL STUDIES TO EVALUATION OF THEIR BIOLOGICAL PROFILES**
	Aula Magna (Department of Pharmacy)	
	Chairs: Maria Laura Bolognesi, Adriano Mollica	
12.10	**PL5: Ana Martinez,** Centro de Investigaciones Biologicas-CSIC, Spain **MODULATING TDP-43 PATHOLOGY WITH PROTEIN KINASE INHIBITORS: A NEW AVENUE FOR ALS THERAPY**	
13.00	**CLOSING REMARKS AND POSTER PRIZES**	
13.30	**LIGHT LUNCH**	

OVERVIEW OF THE SCIENTIFIC CONTRIBUTIONS

Adriano Mollica, Lorenza Marinaccio

Herein, we summarized, in the pictures, the participation and communication contents and their geographical distribution, basing on the accepted contributions. In Figure 1 is represented the geographical distribution of oral and poster communications for each Italian Regions. In Figure 2 is represented the geographical distribution of the Keynotes for each Italian Regions.

Oral (34) and Flash Communications (20 included the two best doctoral thesis awards FCs) and poster presentations (104).

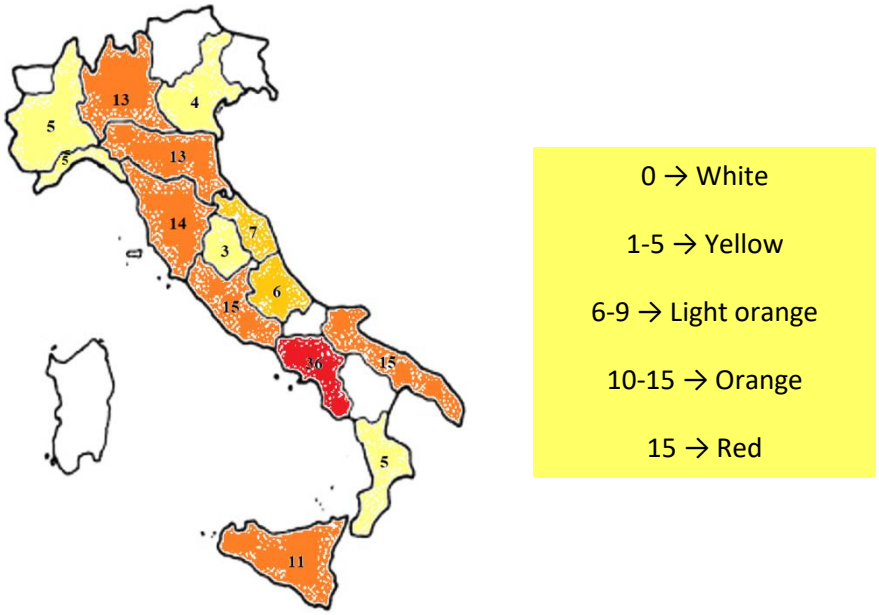

Figure 1. Contributors provenance (Oral and Flash communications and Poster communications), including academic and industrial speakers, by Italian Regions.

Figure 2. Keynotes geographical distribution, including academic and industrial speakers, by Italian Regions.

The international feature of the congress is somehow supported by the 7 foreign contributions (5 plenary lectures and 2 oral/ flash and poster contributions (only the presenting authors have been considered for the analysis).

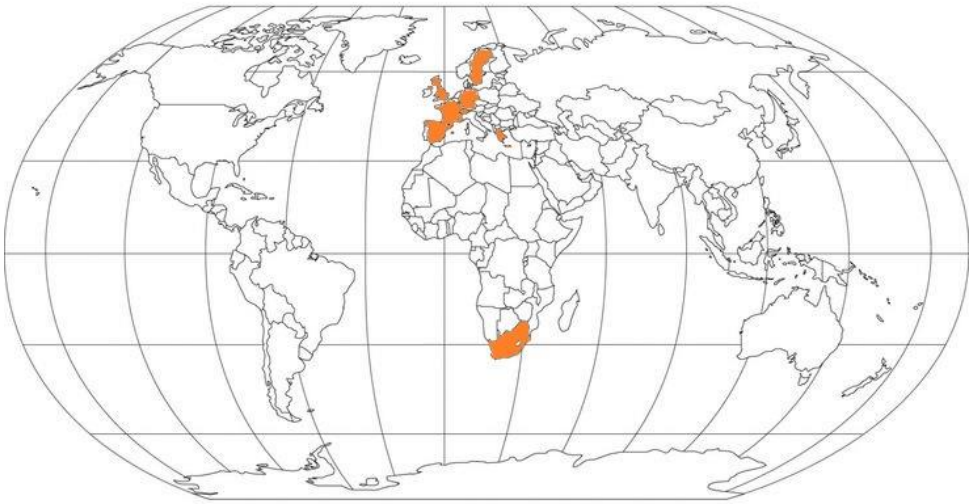

Figure 3. International distribution of the contributions.

The communications contents, are distributed per main topics, as depicted in the bar-graph depicted in Figure 4, with a prevalence of Neurodegenerative disease, Infectious disease and Cancer.

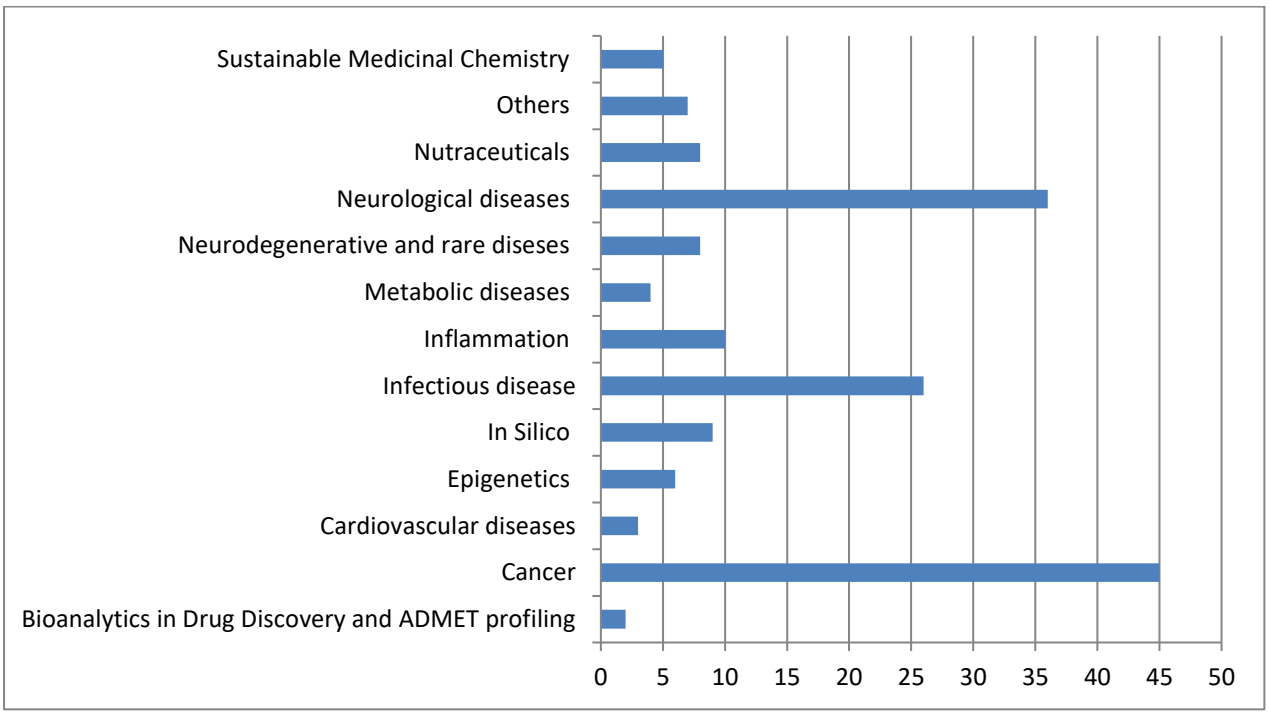

Figure 4. Distribution of the communications per main topics.

PLENARY lecture

PL1

MEDICINAL CHEMISTRY APPROACHES TO REPOSITIONING ANTICANCER HUMAN KINASE INHIBITORS FOR MALARIA

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Drug repositioning, which refers to the chemical modification of a clinically approved drug in one disease area to generate structural analogues optimized for use against another indication, is an attractive approach in drug discovery.[1] Similarities in the cell biology between two cell types can be a rational basis for selecting starting points for drug repositionig. More specifically known drugs that act through protein targets of human origin to identify drugs against malaria parasites with homologous protein targets can be exploited. Within this context, the kinase gene family has been extensively investigated as therapeutic targets in many diseases due to their importance in cellular function. For this reason, these targets are attractive for repurposing in other diseases, including malaria.

Kinase targets essential to multiple stages of the human malaria parasite *Plasmodium* life cycle have emerged as promising drug targets with the potential to deliver antimalarials with multistage activity.[2] Given that human kinases have been targeted extensively in cancer, there is a vast knowledge base to guide the design of inhibitors selective towards the parasite targets. Phenotypic screening of anticancer human kinase inhibitors for antiplasmodium activity, followed by target identification studies have provided novel inhibitor-kinase pairs as starting points for target-based malaria drug discovery and repositioning through medicinal chemistry approaches.[3]

This talk will describe an integrated approach to repositioning anticancer human kinase inhibitors for malaria underpinned by medicinal chemistry using biochemical functional assays, coupled with computer-aided drug design, to optimize hit compounds for both potency against *Plasmodium* kinase targets and selectivity relative to key human off-targets.

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PL2

DEVELOPMENT OF GENERIC G PROTEIN PEPTIDOMIMETICS ABLE TO STABILIZE ACTIVE STATE Gs PROTEIN-COUPLED RECEPTORS FOR APPLICATION IN DRUG DISCOVERY

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G protein-coupled receptors (GPCRs) represent an important group of membrane proteins that play a central role in modern medicine. Unfortunately, conformational promiscuity hampers full therapeutic exploitation of GPCRs, since the largest population of the receptor will adopt a basal conformation, which subsequently challenges screens for agonist drug discovery programs. Herein, we describe a set of peptidomimetics able to mimic the ability of G proteins in stabilizing the active state of the b2 adrenergic receptor (b2AR) and the dopamine 1 receptor (D1R). During fragment-based screening efforts, these (un)constrained peptide analogues of the a5 helix in Gs proteins, were able to identify agonism preimprinted fragments for the examined GPCRs, and as such, they behave as a generic tool, enabling an engagement in agonist earmarked discovery programs.

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PL3

EXPLOITING ALLOSTERY FOR COMPUTER-AIDED DRUG DESIGN TO TARGET ONCOGENES

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A key challenge in targeting mutated proteins (oncogenes) with small molecule inhibitors is to selectively target only the mutated protein and leave the wild-type (WT) protein unaffected, thus avoiding undesirable side effects. One of the problems with selective targeting of mutant proteins with small molecules is that the mutation occurs far from the active site of the protein at a distant location, where druggable cavities are usually not characterized. However, these distal sites may transmit signals to the active site or to other functional sites on the protein through allostery, i.e. networks of amino acids that communicate information between different parts of the protein. These networks are called allosteric pathways, allowing for regulation of protein activity. A working hypothesis is that at the allosteric sites that transmit signals to protein functional sites druggable cavities exist, which can accommodate small molecule modulators of protein function. Allosteric modulators typically bind to less conserved sites compared to the active site of an enzyme, and thus they may confer greater specificity in mutant proteins compared to the WT, or selectively target a specific isoform within a protein family.[1,2] Moreover, proteins that do not have a known active or functional site and are considered undruggable, such as KRAS, could be targeted with allosteric modulators. In this work, we investigate the mechanism of over-activation of oncogenic mutants, including the H1047R and E545K hotspot mutants of PI3Kα[3,4] and the G12D mutant of KRAS.[5] We calculate allosteric pathways and show residues important in delivering communication signals between functional domains of each protein. Taking into account these results, we investigate opportunities for allosteric drug design.[6,7]

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PL4

APPLICATION OF THE CELLULAR THERMAL SHIFT ASSAY IN DRUG DISCOVERY

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Confirmation of compound binding to target proteins of clinical importance plays a central part in drug discovery projects. This "target engagement" is monitored in increasingly complex model systems, ranging from isolated recombinant proteins to whole organisms, with the aim to tie these interactions to disease-relevant pharmacology. Such validation of pharmacologically active molecules, and their mechanism-of-action, is essential as it reduces clinical attrition for candidate drugs.

Traditionally target engagement has been measured indirectly through the use of proximal biomarkers, where physical binding of drugs to target proteins can be inferred from downstream markers of pharmacological response. With the introduction of a repertoire of more direct target engagement methodologies, such as the cellular thermal shift assay (CETSA), we can now complement these approaches with measurements of drug interactions inside live cells and tissues.

This presentation will provide a historical overview of how the Swedish innovation CETSA became a well-respected methodology for validation of chemical probes in academic research, while also finding its way into pharmaceutical industry. An overview of drug discovery applications will be provided, with details from a 0.5 million compound screen at AstraZeneca using high throughput CETSA to generate new chemical matter for the notoriously challenging oncology target cRAF. Results will also be presented from systematic experiments to translate CETSA measurements into quantitative interpretation of binding affinities and the application of CETSA to integral membrane-spanning proteins.

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PL5

MODULATING TDP-43 PATHOLOGY WITH PROTEIN KINASE INHIBITORS: A NEW AVENUE FOR ALS THERAPY

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TAR DNA binding protein of 43 kDa (TDP-43) is a highly conserved nuclear protein that regulates RNA metabolism and the expression of multiple genes, controlling the production of a great number of proteins. In TDP-43 proteinopathies, such as is the case of neurodegenerative diseases mainly amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD-TDP), the homeostasis of TDP-43 is disrupted. Due to different causes, largely unknown, but including genetics, environmental factors and aging, TDP-43 abandons the nucleus and moves to the cytoplasm where suffer different post-translational modifications, mainly hyperphosphorylation, that reduce its solubility forming toxic aggregates. This fact together with the lack of nuclear function produce TDP-43 pathology. Thus, the recovery of TD inhibitors P-43 homeostasis is an emergent therapeutic approach for the treatment of unmet TDP-43 proteinopathies.[1]

Based in our great experience on design and development small molecules targeting protein kinases for CNS diseases, we have explored the therapeutic potential of CK-1δ, CDC-7, TTBK1 and GSK-3β inhibitors for the potential treatment of ALS.[2-5] These kinases are all of them involved in TDP-43 phosphorylation *in vivo*. Following a reverse chemical genetic approach and subsequent hit-to-lead medicinal chemistry programs, we have now focused chemical libraries of brain penetrant and selective inhibitors. The better candidates from these series emerge as valuable drug candidates able to restore TDP-43 functional homeostasis. All of them are able to decrease TDP-43 hyperphosphorylation and aggregation, recovering its nuclear localization and function. Moreover, we have also developed a valuable platform to study TDP-43 pathology based in human cells. Lymphoblasts from ALS and/or FTD patients, genetically characterized, recapitulate TDP-43 proteinopathy offering possibilities for personalized medicine.[6] Furthermore, and using this model, we have determined the key role of TDP-43 in cell-to-cell disease spreading. Our kinase inhibitors are also able to stop the disease progression.

All these results, together with the *in vivo* motorneuron or cortical neuron preservation in ALS or FTD models, respectively, will be presented. Only future clinical trials will reveal the therapeutic value of TDP-43 modulation by small protein kinase inhibitors to ALS and FTD patients.

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PRATESI MEDAL, MEDICINAL CHEMISTRY DIVISION - ITALIAN CHEMICAL SOCIETY

THE DREAM OF PAUL EHRLICH

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More than 100 years ago, Paul Ehrlich envisaged the possibility of achieving a selective therapeutic intervention by means of "magic bullets" (*Zauberkugeln*): molecules capable of selective localization at the site of disease, helping spare normal tissues from undesired toxicity.
The dream of Paul Ehrlich has become closer to reality thanks to the conjugation of bioactive payloads (drugs, radionuclides, cytokines) to suitable protein ligands (antibody fragments or small organic molecules), capable of high-affinity binding to accessible protein targets, selectively expressed at the site of disease.
The discovery of human antibodies and of small organic ligands has been greatly facilitated by advances in encoded combinatorial library technology[1].
In this lecture, I will show how encoded combinatorial libraries can be used to isolate high-quality antibodies and small organic ligands for *in vivo* tumor targeting applications[2]. I will also show how these building blocks can be used for the creation of novel biopharmaceuticals, which are exhibiting promising results in advanced clinical trials in patients with cancer.

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KEYNOTE lectures

KN1

THE CONTRIBUTION OF PEPTIDE RESEARCH TO THE FIGHT AGAINST SARS-COV-2: FROM VACCINES TO ANTIVIRALS

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The devastating pandemic of severe acute respiratory syndrome known as COVID-19 is caused by the coronavirus SARS-CoV-2. A trimeric spike (S) protein expressed on the virus outer bilayer leaflet has been identified as a ligand that allows the virus to penetrate human host cells and cause infection. Its receptor-binding domain (RBD) interacts with the angiotensin-converting enzyme 2 (ACE2), the host-cell viral receptor, and therefore the interaction between these two proteins is the subject of intense research for the development of virus control means, both vaccines and antiviral drugs.

In a first research line,1 we searched for smaller fragments of the S protein able to elicit virus-neutralizing antibodies, suitable for production by peptide synthesis technology. On the basis of structural data, we prepared a synthetic 72-mer peptide, containing the binding motif of RBD. We subsequently used an immunoenzymatic assay (ELISA) to study the antibody response of this peptide, in comparison with recombinant proteins S and RBD, in humans exposed to the infection and in immunized mice. The sero-reactivity analysis showed that anti-peptide antibodies are produced in COVID-19 patients and immunized mice and may exert neutralizing function, although with a frequency lower than anti-S and anti-RBD. These results provide a basis for further studies towards the development of synthetic vaccines focused on specific regions of the S virus protein, which can benefit from the absence of folding problems, conformational constraints, and other advantages of the peptide synthesis production.

We also implemented a strategy based on synthetic peptide to develop new antiviral compounds mimicking the binding motif of ACE2 and potentially able to inhibit the ACE2/Spike protein interaction, thus preventing the cell entry.2 As most of the ACE2 residues involved in the interaction with the viral S protein belong to the α1 helix, we focused our attention on the minimal fragment ACE2(24-42) and, in order to increase the stability of the secondary structure, we designed different triazole-stapled analogs, changing the position and the number of bridges. The peptide featuring a triazole-containing bridge linking positions 36-40 showed increased helical conformation, as determined by circular dichroism, and promising antiviral activity at micromolar concentration, assessed by plaque reduction assay.

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KN2

DESIGN AND DEVELOPMENT OF NEW SOLUBLE EPOXIDE HYDROLASE INHIBITORS AS PHARMACOLOGICAL TOOLS IN INFLAMMATORY DISEASES

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The present lecture is based on the scientific efforts made by my research group in the search of new chemotypes acting as anti-inflammatory drugs. It is well-known how arachidonic acid cascade involves different actors playing both anti-inflammatory and pro-inflammatory roles. The severity and the course of inflammation strictly depends on the balance between these mediators. Soluble epoxide hydrolase (sEH) has gained huge attention because of its role in the hydrolysis and consequent deactivation of the pro-resolving cis-epoxyeicosatrienoic acids (EETs). Thus, development of sEH inhibitors is considered as a new and profitable approach in the treatment of inflammatory disorders.[1] Recently, by mean of a collaborative and multidisciplinary research group, we have identified some indoline-based compounds acting both as 5-lipoxygenase (5-LOX) and sEH inhibitors. These derivatives showed a suitable in vitro and in vivo pharmacokinetic properties that mostly supported the efficacy shown when administered in vivo models of peritonitis and asthma.[2] Moreover, the structure-activity relationships regarding this group of compounds allowed the identification of the structural determinants driving their multi-target activity. In addition, we were also able to identify some specific structural elements able to switch the compounds selectivity over the sEH. To verify this hypothesis a series of indole-based compounds were designed as selective sEH inhibitors. These molecules showed high selectivity and potency in sEH inhibition together with in vivo efficacy in a murine model of acute pancreatitis. Their activity is strictly dependent on sEH inhibition as shown by lipidomic in vivo analyses. These results pave the way for the future development of potent and safe new anti-inflammatory agents.

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KN3

MEDICINAL CHEMISTRY OF INHALED DRUGS: EXAMPLES FROM VALIDATION TO PRECLINICAL CANDIDATES

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The use of an adequate tool compound is of pivotal importance in evaluating the therapeutic potential of a biological target in the context of target tractability and translability1. Beyond on-target potency and selectivity, a tool compound is needed to have sufficient pharmacokinetic properties, and exposure at site of action, to allow in vivo use in experimental models of disease. When considering inhalation route to treat lung diseases, it is necessary a tool compound that have an adequate lung to plasma split in order to dissect local vs systemic contribution to correctly evaluate on-target tractability. Under the guidance of inhaled by design principles2, we will show some examples of inhalatory optimization aimed at delivering tool compounds suitable for use in chronic models of respiratory disease of interest. The main case study of this communication will describe the medicinal chemistry optimization of a series of Rho Kinase inhibitors for inhaled administration used in the area of pulmonary artery hypertension (PAH)3.

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KN4

IN SILICO POLYPHARMACOLOGY STUDIES FOR CNS CONDITIONS

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The emergence of network pharmacology raised expectations for a new era in drug discovery, with the anticipation that *-omics* data would facilitate the identification of unprecedented and non-trivial target combinations.[1] The envisioned outcome was the design of novel molecular entities capable of targeting these combinations. However, the realization of this potential has faced challenges. In this study, we present a systematic effort to compile a dataset of recently reported Dual Target-Directed Ligands (DTDLs), the simplest form of multitarget-directed ligands. Through our investigation, we aim to assess the impact of the targets' structure, function, and chemogenomic traits on the synthetic outcome. Within this framework, we will discuss recent advancements in our work on DTDL series, which we have been studying for several years. Encouraged by positive results generated in an *in vivo* model of nicotine addiction, we will report how insights gained through dual dopamine receptor D3 (D3DR) – Fatty Acid Amide Hydrolase (FAAH) modulators eventually led to the discovery of a new series of D3DR – PPAR-a DTDLs.[2,3] Furthermore, we will explore how molecular modeling studies are advancing the development of D3DR – GSK-3β DTDLs, molecules holding potential for the treatment of bipolar disorder.[4]

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KN5

APPLICATIONS OF PROTEIN-BASED ANALYTICAL METHODS IN DRUG DISCOVERY

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Bioanalytical tools based on soluble proteins or solid-supported proteins integrated into analytical platforms could be crucial for screening new ligands, probing ligand-target interactions, and monitoring binding events through on-line (1D, 2D, or both) or off-line methods.

The way to immobilize a biological target onto a solid surface is the cornerstone for developing many bioanalytical assays (i.e., biochromatography or magnetic beads) while, when working with a free biological target in solution, a suitable ligand fishing method is required to recover the ligand-target complex from the solution. The isolation of the complex is based on the difference in size or mass of the complex with the remaining elements of the complex matrix (i.e., ultrafiltration membranes or size exclusion chromatography).

Peroxisome Proliferator-Activated Receptors (PPARs) are key nuclear receptors and therapeutic targets for the treatment of metabolic diseases through the regulation of insulin resistance, diabetes, and dyslipidemia. Although a few drugs that target PPARs have been approved, more diverse and novel PPAR ligands are necessary to improve the safety and efficacy of available drugs.

Different affinity-based methods using immobilized PPARs will be described and pros and cons of the different approaches in ligand discovery initiatives will be discussed. In addition, the development of a new affinity in solution assay towards natural extracts will be presented.

Natural products have been an important source for the discovery and development of new ligands that target PPARγ, whereas only a few natural ligands of PPARα have been reported to date. In this regard the identification of novel ligands of PPARα or PPARγ from complex extracts of dietary foods or botanicals requires a sensitive and selective screening assay to increase throughput. To expedite the search for new natural ligands of PPARs, an innovative screening assay based on size exclusion chromatography coupled to liquid chromatography-mass spectrometry (2D-SEC-RP-MS) has been developed. The automated bidimensional affinity-selection mass spectrometry screening assay has been validated with known ligands and applied to different extracts.

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KN6

SMALL MOLECULES INTERFERING WITH HUR–RNA COMPLEXES: A PROMISING STRATEGY TO DISCOVER ANTICANCER AGENTS

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Embryonic Lethal Abnormal Vision (ELAV) proteins are highly conserved RNA Binding Proteins (RBPs) that, in response to intra- and extra-cellular signals, preferentially bind to definite target mRNAs, increasing the cytoplasmic stability and/or the rate of translation and promoting gene expression. Among RBPs, HuR is one of the proteins involved in modulating the expression of various oncogenes and tumor suppressor genes, playing a role in tumorigenesis, i.e. cell proliferation, angiogenesis and metastasis (Figure 1). Compounds able to modulate the complex HuR–RNA represent a new opportunity for fighting cancer[1].
The complexity of HuR's role and function represents the first challenge towards its pharmacological modulation, which requires a careful evaluation of the biological context in which a given molecule acting on HuR–RNA complex can be tested/used. A second major challenge is the identification of small molecules able to directly interact with HuR. Indeed, although several studies have identified the protein domains that interact with target mRNAs, little is known about the features of the HuR–small molecule interaction. Lastly, as for the vast majority of RBPs, the mechanism of modulation of HuR by small molecules is still unclear.

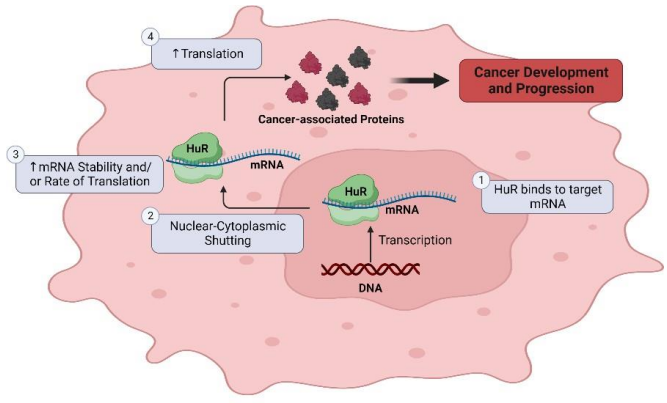

Figure 1. HuR role in regulating target mRNAs.

Therefore, targeting HuR–RNA complexes, and Hu–RNA complexes more generally, is an innovative approach for identifying new biologically active compounds that is gaining increasing consensus among medicinal chemists.
By applying different strategies (in silico studies combined with STD NMR, virtual screening, fragment-based drug discovery, and dynamic combinatorial chemistry), we identified HuR ligands[2,3].
Most compounds bind HuR, interfere with HuR–RNA complexes, and showed antitumor properties in a screening on commercial human cancer cell lines. These compounds represent the starting point for the development of valuable therapeutics, with an innovative mode of action that holds great promise in the fight against cancer.

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KN7

MEDCHEM STRATEGIES TO SELECTIVELY TARGET THE PI3K-MTOR PATHWAY FOR ONCOLOGY APPLICATIONS

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Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered valuable assets in cancer therapy. Despite many pan-PI3K inhibitors have been investigated as anticancer agents, most of them failed in clinical trials due to on-target metabolic side effects such as hyperglycemia and hyperinsulinemia. To overcome the limitations of pan-PI3K inhibitors we developed (i) highly selective mTOR inhibitors, and (ii) selective PI3K covalent molecules.

In 2018, we discovered PQR620, the first-in-class brain-penetrant ATP-competitive mTOR inhibitor (TORKi) able to attenuate epileptic seizures in a mouse model of Tuberous Sclerosis Complex (TSC).[1] Despite promising results in rodent disease models, the limited stability of PQR620 in human hepatocyte assays and short half-live in pharmacokinetic studies in Cynomolgus monkeys, prevented its entry into clinical development. Aiming to develop follow up compounds for PQR620, we have disclosed a conformational restriction strategy and discovered the first pyrimido-pyrrolo-oxazine highly selective TORKi (PQR617).[2] While the first-generation tricyclic compounds displayed a limited brain penetration, investigation on the heteroaromatic ring led to second generation pyrimido-pyrrolo-oxazines with predicted BBB permeability.[3] In addition, we combined pharmacophore features of PQR620 and PQR617 to identify PQR626. PQR626 displayed an excellent brain penetration, very good tolerability in mice and was able to significantly prevent mortality in Tsc1GFAPCKO mice.[4] Recently, we explored 3,6-dihydro-2H-pyran and tetrahydro-2H-pyran as isosteres of the morpholine moiety to unlock a novel chemical space for TORKi generation.[5] Overall, we exploited a variety of chemical strategies to identify metabolically stable mTOR inhibitors for the treatment of cancers and neurological disorders.

In parallel, we exploited a rational approach to increase target selectivity by covalently targeting PI3Kα at the non-conserved nucleophilic Cys862. The development of isoform-selective covalent compounds represents a major step towards an increased local and temporal control of PI3K in precise and innovative cancer therapy.[6]

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KN8

MIDAS: A COMBINED ENHANCED SAMPLING MOLECULAR DYNAMICS AND FRAGMENT-BASED APPROACH TO LOCATE ALLOSTERIC BINDING SITES IN G PROTEIN-COUPLED RECEPTORS

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G protein-coupled receptors (GPCRs) are the most sought after drug targets. Conventional drugs bind to the orthosteric binding site, that is conserved within sub-family members. As such, drugs often bind to several GPCRs mediating different responses, thus leading to side-effects. To achieve sub-type selectivity and reduce side-effects spatially distinct sites, *i.e.*, allosteric binding sites (ABSs), can be targeted with allosteric modulators (AMs). Until recently, ABS location in GPCRs was largely unknown. Recent structural biology breakthroughs yielding GPCR structures complexed with AMs opened new avenues to develop computer-aided strategies to identify ABSs and to enable the structure-based design (SBD) of AMs.

I hereby present MIDAS, a novel molecular dynamics (MD)-based approach[1] to locate ABSs at GPCR solvent-exposed and the membrane-facing sides (Figure 1). The protocol exploits *cylinder-shaped potentials* applied to *privileged fragments* derived from known AM structures to identify key amino acid residues forming the ABSs. MIDAS was validated *retrospectively* in three case studies and demonstrated to outperform classical co-solvent MD by enhancing probe sampling in the ABSs. MIDAS was validated also *prospectively* to locate the ABS of a known D2 dopamine receptor AM with potential application to treat Parkinson's disease[2]. Site-directed mutagenesis experiments supported the predictions, thus showing that MIDAS can aid the SBD of novel GPCR AMs while its application can extend to other membrane proteins.

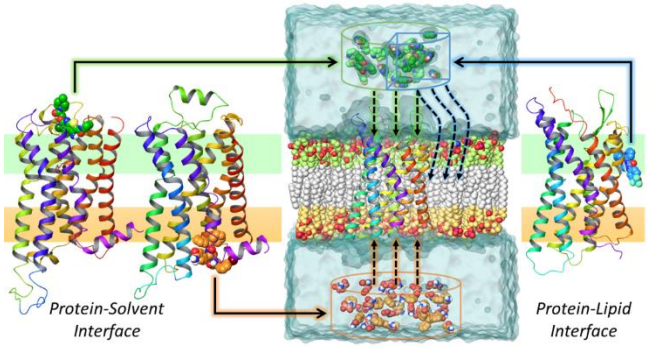

Figure 1. Schematic representation of MIDAS approach.

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KN9

TARGETING FORMYL PEPTIDE RECEPTORS TO TACKLE INFLAMMATION IN NEUROLOGICAL DISORDERS AND CANCER

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Formyl peptide receptors (FPR) are G protein-coupled receptors that play important roles in host defense and inflammation. After their discovery, it was assumed that these receptors predominantly govern a pro-inflammatory response resulting in chemotaxis, degranulation, and oxidative burst during infection.[1] It is now becoming clear that the activation of FPRs has more complex consequences and can promote the resolution of inflammation.[2] Accumulating evidence demonstrates that FPRs are involved in several pathophysiological processes, including neuroinflammation, angiogenesis, and tumor growth, and participate in disease initiation, progression, and resolution.[3] The development of agonists and antagonists for FPRs will be illustrated, and the therapeutic potential in addressing neuroinflammation and cancer will be discussed.

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KN10

THE DISCOVERY OF I-0436650, A POTENT AND SPECIFIC ALLOSTERIC SHP2 INHIBITOR FOR THE CANCER THERAPY

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Inhibition of SHP2 (Src homology-2 domain-containing protein tyrosine phosphatase-2) is a promising therapeutic strategy to target RAS-driven cancers and to modulate immune signaling pathways within the tumor microenvironment[1]. The first report of a small molecule capable of inhibiting SHP2 activity through an allosteric mechanism triggered the development of various SHP2 inhibitors which are currently in clinical development, both in monotherapy and in combination therapy[2].
Structure-based de novo design efforts led to the imidazopyrazines series[3] which pioneered the identification of novel azabicyclic[4] SHP2 inhibitors. From comprehensive SAR studies, I-0436650 emerged as a remarkably well-balanced compound across a multitude of pre-clinical attributes. I-0436650 is a potent and specific SHP2 allosteric inhibitor which can suppress the intracellular RAS/MEK/ERK signaling pathway in a dose dependent manner. I-0436650 exhibits significant antiproliferative activity against multiple RAS and EGFR mutant cancer cell lines *ex-vivo* and inhibits tumor growth *in vivo* in mouse xenografts models of cancer.

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ORAL communications

OC1

GREENING THE SOLID-PHASE PEPTIDE SYNTHESIS: FURTHER CHALLENGES TO IMPROVE SUSTAINABILITY IN THE DEVELOPMENT OF THERAPEUTIC PEPTIDES

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The development of greener synthesis processes is a real necessity to transform the industrial landscape, especially the pharmaceutical sector, into a more sustainable reality. Due to the increasing demand from the chemical and pharmaceutical markets for synthetic peptides in the last years, the attention to the greening of their production represents a significant challenge droving researcher toward the introduction of sustainable processes to prepare highly pure, active pharmaceutical ingredients (APIs).[1]

Nowadays the preferred method to obtain peptides is the solid phase peptide synthesis (SPPS), which, unfortunately, does not respect the principles of green chemistry due to the large amount of toxic reagents and solvents used. Because the synthetic procedures do not admit a reduction in the amount of solvent, several attempts have been reported in recent years for replacing DMF with greener solvents,[2,3] according to well-known solvent-selection guides. In this contest, we report a study focused on the replacement of DMF in the fluorenyl methoxycarbonyl (Fmoc) solid-phase peptide synthesis with Dipropylene glycol dimethyl ether (PROGLYDE™, DMM), a well-known green solvent with low human toxicity following oral, inhalation and dermal exposure and easily biodegradable. Some tests needed to evaluate its applicability to all the steps of the SPPS such as amino acid solubility, resins swelling, kinetics deprotection and coupling tests. Once the best green protocol was established, it was applied during the synthesis of a different length peptides to study some of the fundamental parameters of green chemistry, PMI (process mass intensity) and recycling of solvent.

In summary, we can conclude that the use of DMM does not impair the synthetic process and that its adoption in the synthetic scheme will be translated into less impact on the environment and the human health.

Figure 1. Greening the SPPS.

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OC2

RECENT ADVANCES IN THE DISCOVERY OF HEME OXYGENASE-1 INHIBITORS WITH ANTICANCER ACTIVITY

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The overexpression of the inducible isoform of the heme oxygenase family, namely HO-1, has been reported as a well-established strategy adopted by cancer cells for the activation of several cellular pathways promoting antiapoptotic effects and chemoresistance.[1] The aberrant expression of HO-1 in tumor cells put a spotlight on the potential anticancer effects attributable to its selective inhibition. In this regard, our research group has been deeply involved in the design of potent HO-1 inhibitors throughout the last decades.[2] Among the compounds recently discovered by us, *N*-methylacetamide and oxybutylimidazole derivatives (Figure 1) displayed promising HO-1 inhibitory potency.[3,4] Additionally, the *N*-methylacetamide moiety led to the identification of VP18/58 (HO-1 IC_{50} = 0.95 µM; HO-2 IC_{50} = 1.2 µM), a potent compound which outstand for its *in vitro* anticancer effects in a model of glioblastoma. In this communication, we report our latest advances in the discovery of novel HO-1 inhibitors derived from the aforementioned chemical scaffolds. Specfically, with the aim of ameliorating the drug-likness of the *N*-methylacetamides, we focused on bioisosteric replacement of the amide moiety. On the other hand, oxybutylimidazole derivatives have been further optimized using computational approach. The design, synthesis, structure-activity relationships and preliminary biological results of the new sets of compounds will be outlined at the meeting.

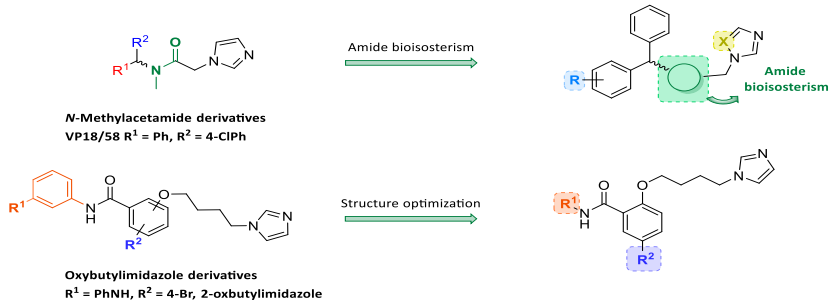

Figure 1. General structures and optimization strategies for the identification of novel HO-1 inhibitors.

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OC3

DISCOVERING ALZHEIMER'S Aβ-AMYLOID FIBRILLOGENESIS INHIBITOR PEPTIDES THROUGH A COMBINED 3D-GRID PHARMACOPHORE AND METADYNAMICS STRATEGY

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The pathogenic aggregation of the amyloid β-peptide (Aβ) is considered a hallmark of the progression of Alzheimer's disease (AD), the leading cause of senile dementia and one of the principal causes of death.[1] Consequently, the inhibition of the early stages of Aβ aggregation is considered a promising approach to treat AD, though structural insights into the nature of Aβ aggregates are elusive, making peptide-drug design difficult.[1] In the last decades, several short synthetic peptides were designed as beta-sheet breakers (BSBPs). Among them, the peptide Ac-LPFFD-NH_2 (iAβ5p),[2] despite the reduced *in vivo* amyloid deposition, it showed a relatively short *in vivo* half-life.[2] For this reason, the incorporation of N-methyl amino acids was subsequently introduced with the double aims to prevent endoproteolytic degradation and to break the peptide-peptide hydrogen bonding interactions.[3] On the basis of such observations, we have designed synthesized and biologically evaluated a small library of N-Methyl peptides. To this aim, 3D GRID-based Pharmacophores were built and the unbiased conformers generation leading to the pharmacophore models of the most promising BSBPs was compared with that found by enhanced sampling Metadynamics calculations. Our combined approach led us to retrieve the cell-permeable peptides 2 and 3 as the most promising new BSBPs endowed with the ability to inhibit fibrillogenesis by ThT binding.

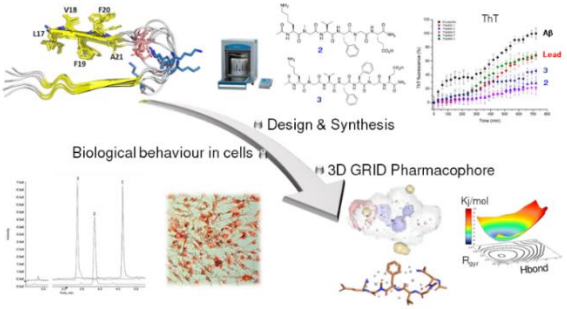

Figure 1: General scheme of the rational peptide-design approach adopted in this work.

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OC4

A FLEXIBLE SPATIAL-MASS SPECTROMETRY STRATEGY APPLIED TO TRAUMATIC BRAIN INJURY AND ITS ASSOCIATION WITH ALZHEIMER'S DISEASE

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The comprehension of physio-pathological mechanisms in various neurological diseases is a challenging task and relies on the development of advanced bioanalytical technologies.

Mass spectrometry, with its ability to simultaneously map hundreds of compounds, can support the understanding of the mechanisms of neurodegeneration, but conventional MS-strategies are limited due to sample-homogenization.

MALDI Imaging-based spatial-omics, on the other hand, allows to investigate the modulation of biomolecules in the context of the tissue geometry and their dynamic spatial distribution.[1]

The present work shows the application of a MALDI-mass spectrometry imaging strategy in a murine model of neurological disease namely mild traumatic brain injury (mTBI)[2].

All experiments were carried out on 9-18 weeks-old male wild-type C57BL/6J (WT-sham, n=3) and APP transgenic Tg2576 mice (APP-sham, n=3). The mild traumatic brain injury (WT-mTBI, n=3 and APP-mTBI, n=3) was induced through the "weight drop model" to replicate a diffuse traumatic brain injury (DTBI) without focal lesion.

The MALDI-MSI approach was based on the combination of two HRMS-analyzers and different matrices, obtaining the simultaneous characterization of several neurotransmitters and metabolites, allowing to precisely evaluate their modulation in specific brain regions associated with the effects of mTBI on the subsequent development of Alzheimer's disease-related neuropathology (AD) and cognitive impairments.

A significant and regio-specific alteration of the endogenous tone of serotoninergic neurotransmitters endocannabinoids and free fatty acids was revealed in both Wild-Type (WT) and AD mice after TBI occur.

These results underline the potential of MALDI-MSI-based approaches as a complementary tool to characterize the mTBI-mediated long-term consequence, exploring potential biomarkers that might be partly linked to the biochemical and behavioral dysfunctions of this condition and providing further support for the development of novel therapeutic approaches.

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OC5

A DUAL-TARGET APPROACH FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), a neurodegenerative disorder characterized by gradual cognitive decline and neuronal deterioration, is the most common cause of dementia and is increasingly recognized as a major public health challenge in light of the increasing life expectancy of the population. Unfortunately, therapeutic options for the treatment of AD are still limited to symptomatic medications, which include acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and memantine, an NMDA receptor antagonist. The single-target approach, i.e., the concept of "one molecule, one target, one disease," has been the widely accepted paradigm in the pharmaceutical industry. However, the multifactorial and sporadic nature of AD suggests going beyond the single-target approach and thinking of a multitarget drug that can act simultaneously at different levels.[1] Type 2 cannabinoid receptor (CB2R) agonists have demonstrated their beneficial effect on AD progression by regulating neurogenic inflammation, preventing microglial activation, and avoiding induced cognitive impairment, and their use "in combination" with acetyl or butyrylcholinesterase inhibitors has already been documented in the literature.[2,3] Based on our expertise in the synthesis of selective CB2R ligands and following a merging approach, we designed and synthesized a small library of hybrid molecules combining the pharmacophoric moiety of donepezil and rivastigmine with that of potent and selective CB2R agonists that we developed in-house.[4,5] Among the new compounds, COR1772 showed the most intriguing profile in both in vitro and in vivo assays.

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OC6

THINNED APPLE POLYPHENOLS ALLEVIATE INFLAMMATION IN A MOUSE MODEL OF DNBS-INDUCED COLITIS: LABEL-FREE QUANTITATIVE PROTEOMICS STUDIES

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Ulcerative colitis (UC), one of the major inflammatory bowel diseases (IBDs), is a complex and multifactorial disease whose incidence rate has significantly increased in recent decades. Current available drug therapies, including NSAIDs and corticosteroids, partially relieve the symptoms and are associated with nonnegligible side effects. Hence the search for new therapeutic strategies that counteract inflammation with reduced side effects is still a medical need. Since oxidative stress and inflammation contribute to tissue damage during colitis, the administration of natural compounds with antioxidant and anti-inflammatory activity represents a promising treatment for UC. We recently identified thinned apples (TA), a waste-product of the apple production chain, as a valuable source of polyphenols (TAP) acting as anti-inflammatory and antioxidant agents in a cell model of inflammation[1]. The aim of the present work is to evaluate the therapeutic potential of TAP in a mouse model of DNBS induced colitis. Using state of the art proteomic techniques (LFQ) we performed *ex vivo* studies to describe at the molecular level the pathological phenotype and the proteins modulated following treatment with the TAP extract. Overall, proteomics studies allowed the identification of 5400 proteins and delineated the molecular pathways evoked by TAP treatment (figure 1): (i) activation of antioxidant-acting mechanisms; (ii) reversal of mechanisms overexpressed/activated in the presence of DNBS, with particular reference to mechanisms of ferroptosis and heme-toxicity; (iii) inhibition of the immune response; (iv) reduced ulcerative condition with a consequent downregulation of proteins involved in the coagulation, inflammation and angiogenesis processes. These results suggest that TAP can be considered a valuable source of polyphenols for health care products to prevent/treat oxidative and inflammatory chronic conditions in UC; moreover, TA represent an innovative source for the industrial production of bioactive extract.

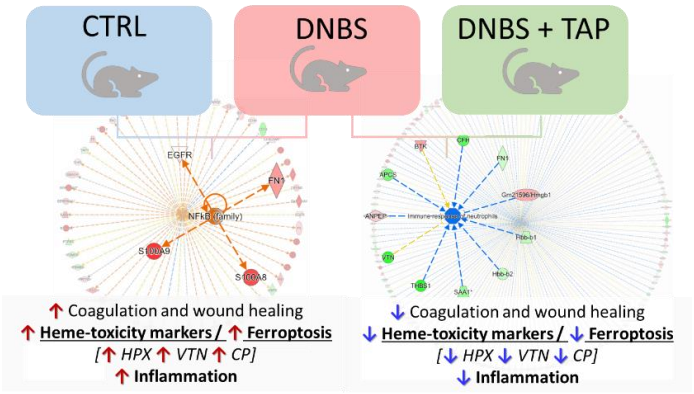

Figure 1.

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OC7

DONEPEZIL-LIKE DUAL TARGET AChE AND MAO-B INHIBITORS WITH PHOTOMODULABLE INHIBITORY ACTIVITY

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Neurodegenerative disorders are multifactorial diseases characterized by neuronal loss and cognitive dysfunctions. Novel agents able to address more than a single event at the same time, i.e., multitarget-directed ligands (MTDLs), have been the focus of promising strategies to disclose new treatments for neurodegeneration, including Alzheimer's disease (AD).[1] Nowadays, pharmaceutical research is increasingly aiming towards precision medicine by attempting to design highly desirable "ad personam" drugs. In this direction, photopharmacology, based on bioactive molecules that can change structural and pharmacological properties as a result of light irradiation, can allow the development of future customizable drugs.[2] Combining our background in medicinal chemistry of MTDLs[3] with the experience in the light-controlled molecular switches,[4,5] we designed a series of photoisomerizable donepezil-like compounds with on-off inhibitory activity towards AChE and MAO-B, both AD-related enzyme targets.[6] The compounds, obtained as *E* isomers, showed an interesting activity toward both target enzymes with IC_{50} values from low micro to nanomolar and a remarkable isoform selectivity. In addition, the irradiation by UV light of *E* isomers provided photostationary states constituted of an excesses of *Z* form and a significant (over one order of magnitude) modulation of the pharmacological properties. Molecular docking revealed the main interactions underlying the different inhibition potency displayed by *E* and *Z* isomers. These results open the way for developing innovative multitarget photoswitchable drugs against neurodegenerative diseases.

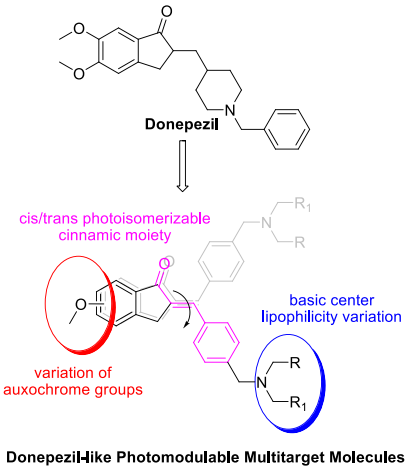

Figure 1. Design strategy of Donepezil-like photomodulable multitarget AChE/MAO-B inhibitors.

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OC8

FYN/GSK-3β DOUBLE KINASE INHIBITORS TO INVESTIGATE NEUROINFLAMMATORY PROCESSES IN NEURODEGENERATIVE DISEASES

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Phosphorylation represents one of the most common post-translational modifications through which kinases can finely tune protein function and signal transduction depending on the (patho)physiological condition. Particularly, in neurodegenerative diseases GSK-3β and Fyn's kinase activities play a crucial role by triggering and fostering neurotoxic inflammatory and protein misfolding processes. In addition, both kinases are physiologically involved in synaptic plasticity and glia formation. Furthermore, Fyn inhibition was accounted for reducing glial activation and misfolded protein-mediated neurotoxicity, whereas a decreased formation of neurotoxic protein aggregates and regulation of inflammatory responses were related to GSK-3β inhibition.[1,2] In this context, we envisioned the possibility to develop new double kinase inhibitors which can simultaneously suppress Fyn and GSK-3β activities to achieve polyhedral neuroprotective and immunomodulatory agents.

Starting from a virtual screening campaign we have previously identified the 7-azaindole-3-aminothiazole scaffold able to inhibit both kinases at micromolar level. From this bioactive core and after several rounds of computer-aided chemical optimizations we obtained potent selective or balanced kinase inhibitors with activities in the nanomolar range, allowing us to better define chemical space and required interactions for an optimal target inhibition (Figure 1). Biological evaluations of more promising compounds revealed a neuroprotective profile in primary cerebellar granule neurons at concentrations comparable with their kinase inhibitory activities. Furthermore, they proved to stimulate proliferation, growth and polarized differentiation of neural stem cells, resulting in an overall neurotrophic effect. Interestingly, different biological activities were ascribed to the inhibition of the two kinases, resulting in boosted efficacy in the more balanced compounds. Further investigations are still ongoing to evaluate the immunomodulatory properties in glial cells and fully characterize the neuroprotective profile of this class of compounds.

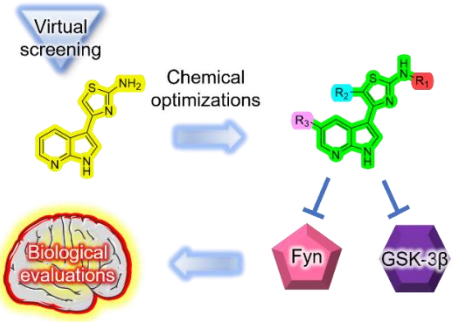

Figure 1. Workflow employed for the identification of new neuroprotective double kinase inhibitors.

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OC9

INTEGRATED FLOW PLATFORM TO STREAMLINE THE SYNTHESIS OF BIOLOGICALLY-ACTIVE STEROIDS

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The global market of steroidal active pharmaceutical ingredients (APIs) and process intermediates is expected to grow up to about $8 billion in 2029,[1] demanding for innovative strategies and improved protocols to meet modern synthesis criteria such as sustainability, safety, and costs. Despite the progress made, steroid chemistry remains challenging and the preparation of steroidal compounds of pharmaceutical interest and in clinical practice often requires long and elaborated synthesis. In this scenario, the application of continuous flow chemistry and related technologies in steroid synthesis and functionalization holds the premise to innovate methodology development and to provide innovative tactics also for many hitherto uncharted chemistries.[2,3] In this communication, we report our ongoing efforts in the development of an integrated and automated flow platform for the synthesis and optimization of high-value steroidal compounds from naturally-occurring and renewable sources. In particular, we showcase the advances made in the field towards more efficient chemical processes for both the manufacturing of steroidal drugs and the preparation of novel chemical entities for medicinal chemistry programs.[4–6]

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OC10

A DYNAMICAL AND ATOMISTIC RESOLUTION VIEW OF THE G PROTEIN COUPLED RECEPTOR A2A ACTIVATION MECHANISM

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G protein coupled receptors are the largest protein family (coded by ~ 4% of human genome) targeted by one third of the currently marketed drugs. Depending on which ligand binds to the receptor, they can assume different conformations activating distinct downstream pathways.[1–5] This phenomenon, known as "biased signalling", is revolutionizing the GPCRs-oriented drug discovery field. In fact, developing molecules capable of selectively stabilizing a certain receptor conformation can activate a specific signalling cascade and yield a tailored control of cell function and treatment of disease.[6,7] In this background, structural insights into the GPCRs functional conformational changes are of paramount relevance. However, although structural data on both the active and inactive endpoints are available, a thorough characterization of the entire GPCRs activation mechanism is still missing. Herein, we developed an advanced free-energy protocol to investigate the GPCR functional and conformational dynamics in cell membrane. We focused on the A2A adenosine receptor, a GPCR involved in several diseases including cancer, inflammatory, cardiovascular, Parkinson's and Alzheimer's diseases. Particularly, we have disclosed at atomistic resolution the sequence of events leading to the GPCR activation, characterizing all the lowest energy - hence most probable - conformational states assumed by the receptor during its action in absence and in presence of agonist and antagonist ligands. Our results reveal a fine allosteric mechanism in which rotation of transmembrane helix 6 and formation of specific inter-helical contacts, namely microswitches, determine receptor activation. The novel structural and energetics data obatined in our study provide the rationale for the design of ligands endowed with biased signaling properties.

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OC11

PK7: A SYNAPTIC TARGETING COMPOUND TO OVERCOME PARKINSON'S DISEASE

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Parkinson's disease (PD), one of the most common neurodegenerative disorder, is nowadays an unmet clinical need, since the gold standard therapy, Levodopa, is efficacious in treating only the motor symptoms but shows adverse effects and lose efficacy with PD progression. We recently identified the neuronal phosphoprotein Synapsin III (Syn III), physiologically cooperating with functional alpha-synuclein (aSyn) to stimulate dopamine release[2], as a key component of aSyn pathologic aggregates[1], supporting that Syn III is pivotal for PD progression and that the pathological aSyn/Syn III interaction could constitute a therapeutic target for PD. Recent literature data suggest how the monoamine reuptake inhibitor methylphenidate (MPH) is able to permit the motor activity recovery in PD tg mice. We demonstrated how this promising activity is related to the re-establishment of the functional interaction between Syn III and α-helical aSyn[3]. Starting from MPH, we developed two generations of derivatives as disease-modifying agents[4a], which were *in vitro* screened using FRET, thus selecting PK7 as our lead compound. PK7, which drug-likeness was predicted, confirmed its ability to positively modulate the aSyn/Syn III complex, did not showed any cytotoxic effect and lost the off-targets effect on MATs, thus avoiding any side effect, common of MPH derived molecules[4b]. Our candidate was able to reduce aSyn aggregates, both *in vitro* and *in vivo*, showed the ability to exert neuroprotection and to restore motor ability[4b]. PK7 efficacy was evaluated also in midbrain organoids from PD patients and the *in vivo* PK/ADME properties were determined[4b], suggesting its strong potentiality for a Phase I clinical development.

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OC12

LEARNING ON HUMAN TYROSINASE: FROM HOMOLOGY MODELLING TO NEW INHIBITORS SELECTION

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Human Tyrosinase (hTYR) represents one of the most targeted metalloenzymes involved in hyperpigmentation disorders.[1] Nonetheless, several studies report its implication in Parkinson disease.[2] Most of the biological assays aimed at identifying novel tyrosinase inhibitors are performed using enzyme from *A. bisporus* (AbTYR), due to its commercial availability. However, structural differences among the various tyrosinases were recently highlighted.[1,3] Highly active inhibitors on AbTYR frequently show reversed activity on hTYR or vice versa. Additionally, a significant challenge in drug development is the lack of experimental 3D-structures for hTYR, increasing the complexity of the task and the rate of false-positives.

Herein, we report a computational study that begins with a retrospective analysis of the biological data of two hTYR inhibitors that showed opposite IC_{50} values for hTYR and AbTYR: the first one is Thiamidol™, a potent and selective hTYR inhibitor;[4] the second one is an in-house molecule that demonstrated high inhibition towards AbTYR [5] and revealed low activity during a screening campaign on hTYR.

We initially developed the hTYR homology model built by softwares (SwissModel, MODELLER, TopModel and AlphaFold), then we performed docking studies and MM-GBSA binding energy calculation for the two inhibitors on hTYR and AbTYR (PDB: 2Y9X). The protocol has led to a rational design that considered crucial structural differences between the two enzymes and resulted in the discovery of a new "hit" exerting dual inhibition at low micromolar concentration. Subsequently, a second part of the work focused on the development of a screening procedure based on the trajectory analysis of MD simulations for Thiamidol™ in hTYR. The *in silico* screening workflow suggested nine potential hTYR inhibitors, that were subjected to biological screening.

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OC13

THE DEVELOPMENT OF HYPOXIA-ACTIVATED PROTACS (HAP-TACS) TO SELECTIVELY DEGRADE BRD4 IN HYPOXIC CANCER CELLS

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Proteolysis targeting chimeras (PROTACs) are bifunctional molecules comprising a ligand for a protein of interest (POI) attached to a ligand for an E3 ligase. They function by inducing degradation of the POI through the ubiquitin-proteasome system. However, the cellular effects of degrading a whole protein are profound, making strategies to target protein degradation to a given location or context essential.[1] This approach will reduce on-target dose-limiting toxicity, enhancing the therapeutic window for the use of PROTACs against a wide range of POIs. Hypoxia (conditions of lower-than-normal oxygen) is a characteristic of solid tumours that conveys poor patient prognosis, but the chemically reducing conditions found in hypoxia allow it to be targeted with pro-drugs. We have previously applied this strategy to KDAC inhibitors, demonstrating selective activation of the pro-drug in a tumour *in vivo*.[2]

To enable context dependent activation of PROTACs, we have developed a series of hypoxia-activated PROTACs (HAP-TACs). To achieve this, we employed a bioreductive group, never applied before, to mask key features of either a cereblon- or VHL-recruiting PROTAC, rendering them inactive in normal oxygen concentrations (normoxia, 21% O_2). In hypoxia, the bioreductive group releases the active E3 ligase ligand enabling degradation of the POI. As a proof-of-principle, we have applied this strategy to the degradation of BRD4. Our HAP-TACs are stable in normoxic conditions, but release the active PROTAC in hypoxia, leading to selective degradation of BRD4 when oxygen levels are reduced. As the bioreductive group is attached to the cereblon or VHL E3 ligase ligand, this strategy is applicable to any PROTAC that recruits these E3 ligases. This work, therefore, provides an exciting avenue for the development of HAP-TACs, for a range of targets, increasing the likelihood of success in the clinic.

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OC14

LYCOPENE EXTRACTION WITH α-PINENE, NATURAL VOLATILE DEEP EUTECTIC SOLVENT MENTHOL-THYMOL AND EXTRA VIRGIN OLIVE OIL

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The production of tomato derivatives is massive in our Country resulting in a huge amount of tomato waste as seeds, peels and stems. The aim of our work is to re-evaluate these matrices through the extraction of carotenoids, especially lycopene. The extractions have been obtained by UAE technique using green solvents, in particular α-pinene, a natural terpene hydrocarbon distilled from pine gum, and the mixture menthol-thymol, a volatile natural deep eutectic solvent[1], then they were compared with n-hexane extract. We also applied the same methodology using extra virgin olive oil as solvent to test its ability to extract carotenoids. The quantification of lycopene has been conducted through HPLC-DAD following the method developed by Olives Barba *et al.* with some modifications.[2] All the extracts showed a good yield in lycopene content. Thus we decided to focus our attention to the extra virgin olive oil extract to determine an improvement of the antioxidant activity. The results are shown in **Table 1** displaying a significant effect especially in DPPH assay. Cytotoxicity assays have been also performed to test its activity. This preliminary work opens concrete perspectives on the production of enriched foods starting from waste resulting in the reduction of the industrial environmental impact and in the promoting of new food production chains.

	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)
Extra virgin olive oil	0,76	11,66	42,59	24,40
Enriched extra virgin olive oil	7,88	10,60	49,74	27,38

Table 1. Antioxidant activities of Extra virgin olive oil and the enriched extra virgin olive oil in DPPH, ABTS, CUPRAC and FRAP assays. Values are reported as mean of three parallel experiments. TE: Trolox Equivalent.

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OC15

POTENT *HUMAN* DIHYDROOROTATE DEHYDROGENASE INHIBITORS BASED ON THE 2-HYDROXYPYRAZOLO [1,5-*A*]PYRIDINE SCAFFOLD AGAINST ACUTE MYELOGENOUS LEUKEMIA: BE PREPARED TO THE CLINIC

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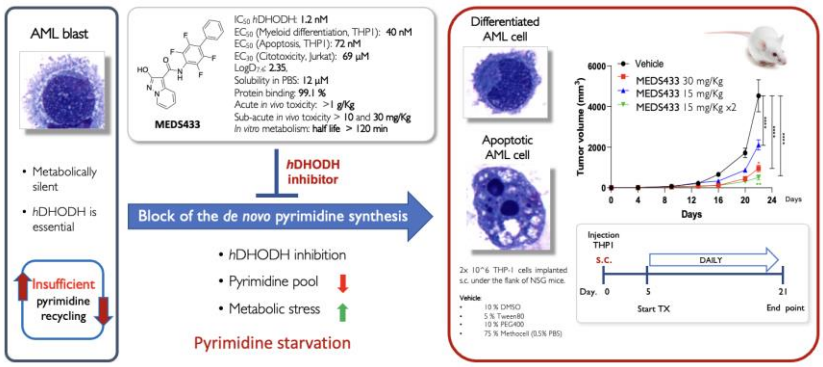
Dihydroorotate dehydrogenase (*h*DHODH), a key enzyme in pyrimidine *de novo* biosynthesis, is considered a novel and attractive target for the *unmet clinical need* Acute Myelogenous Leukemia (AML). By using an innovative bioisosteric approach supported by structure-based techniques, we discovered a new class of *h*DHODH inhibitors based on the 2-hydroxypyrazolo[1,5-*a*]pyridine scaffold. As result of an extensive SAR investigation, **MEDS433** is an advanced preclinical candidate *h*DHODH inhibitor (IC_{50} = 1.2 nM) able to induce myeloid differentiation in AML cell lines in the low nM range (EC_{50} = 40 nM, THP1), superior to the phase I/II clinical *lead brequinar* (EC_{50} = 247 nM, THP1). In this occasion, beside detailing the **MEDS433** SAR, PK, ADME, toxicity (acute/subacute on different species) as well as the *in vivo* efficacy in different models (leukemic xenograft and IV (mouse, IP, PO)), its synthetic technological transfer (8 g batches, purity > 98.5 %) is also presented. To reinforce the scenario the pathway that allowed the discovery of the *backup compound* **MEDS700** (EC_{50} = 17 nM, THP1), as well as the most recent strategies investigated for overcame possible *h*DHODH resistance at clinical level are also detailed. All these studies, most of them still unpublished, are directed to open the incoming **MEDS433** certified preclinical studies necessary for prepare its Phase I clinical trial for AML.



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OC16

DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF HSP90/HDAC6 DUAL INHIBITORS BASED ON A 2-AMINO-PYRROLOPYRIMIDINE SCAFFOLD FOR THE TARGETING OF AGGRESSIVE PROSTATE CANCER

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Prostate cancer (PC) is one the most common types of male cancers, which has been associated to adverse outcomes.[1] Unfortunately, the efficacy of available drugs for the treatment of advanced stage PC remains limited and severely hampered by drug resistance.[1] Novel valuable therapeutic opportunities can arise by the simultaneous inhibition of Hsp90 and HDAC6, two key, interconnected players in aggressive PC forms.[1] In this study, we designed, synthesized and *in vitro* tested several compounds in search of potent Hsp90/HDAC6 dual inhibitors. The design was based on exploration of two different Hsp90 binding pockets and integration of key structural features required for HDAC6 inhibition (Figure 1), leading to two structurally different series of compounds.[1,2] Of note, some of the designed compounds showed nanomolar inhibition of Hsp90 and HDAC6, high selectivity with respect to other HDAC isoforms and potent antiproliferative activities against aggressive PC cell lines. The best candidates of the two series were further characterized for drug-like properties, migration and invasive behavior of aggressive and castration-resistant PC cells, representing valuable candidates for further preclinical investigations.[3,4]

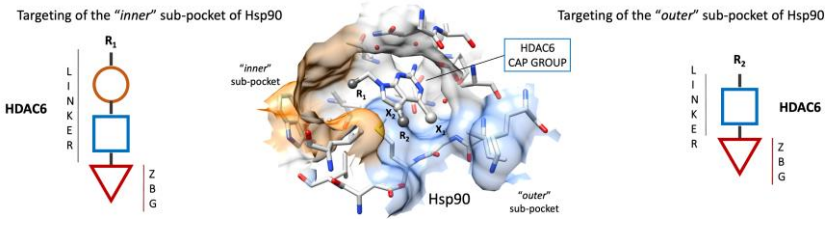

Figure 1: HDAC6/Hsp90 dual inhibitors designed in the study, targeting the "inner" and "outer" sub-pockets of Hsp90.

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OC17

DEVELOPMENT OF A NOVEL MULTI-TARGET ANTICANCER APPROACH BASED ON G-QUADRUPLEX STABILIZATION AND INHIBITION OF HUMAN CARBONIC ANHYDRASES

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Compared to the association of medicines, multi-target-directed ligands (MTDLs) can potentially offer a more predictable pharmacokinetics, reduced risk of drug interactions, and a higher adherence to therapy. In the present investigation, we propose MTDLs hitting two highly promising anticancer targets: G-quadruplex (G4) DNA structures and human carbonic anhydrases (hCAs) IX and XII. G4s are noncanonical four-stranded nucleic acid secondary structures which can arise from guanine-rich regions, including telomeres and oncogene promoters. Induction and/or stabilization of G4s by means of small molecules represent a potential anticancer tool, leading to telomere maintenance problems and reduced oncogene expression.[1,2] Carbonic anhydrases IX and XII are two proteins that have been found to be upregulated in many hypoxic tumors, contributing to an aggressive metastatic phenotype.[3] Inhibition of hCAs IX and XII has been consolidated over the last two decades as an innovative chemotherapeutic strategy against solid (and hypoxic) tumors.[4]
Herein, we synthesized a library of molecular hybrids containing both a well-known G4 stabilizer (berberine) and an inhibitor of hCAs IX/XII as potential multi-target anticancer agents. The *in vitro* ability of the newly synthesized compounds to stabilize G4 structures and inhibit the tumor-associated hCAs IX and XII was assessed. The most promising derivatives were subjected to a further biological characterization, leading to the identification of a hybrid displaying a good cytotoxic effect on CA IX-positive human cervix cancer cells, even greater under hypoxic conditions, as well as the ability to stabilize G4 structures also in the cellular environment.

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OC18

MICROALGAE: BIOACTIVES EXTRACTION AND CHARACTERIZATION FOR NUTRACEUTICAL USAGE

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Microalgae are naturally rich of bioactive compounds of great interest in the preparation of a wide range of products such as food, cosmetics and nutraceuticals.[1-3] The analytical characterization of commercial dried microalgal biomass of Chlorella and Spirulina samples aimed this project to the investigation of their content in terms of micro and macro nutrients. The research was oriented towards the development and validation of accurate, fast and reproducible methods for the nutritional assessment of microalgal biomasses, in order to provide a guiding methodology. The lipid profiles of algal matrixes were analysed regarding to the content of saturated, unsaturated and polyunsaturated fatty acids. First, the total lipids and pigment content was extracted and it was gravimetrically quantified; then, the lipid-pigment portion was trans-esterified to analyse the fatty acid methyl esters with a GC-MS method. A fingerprinting of MUFAs and PUFAs was obtained regarding microalgae species. The determination of total carotenoids and chlorophylls content in the lipid extracts was evaluated through a fast UV-Vis spectrophotometric analysis, which was validated by a new HPLC-DAD analysis. Furthermore, the total antioxidant activity of each lipid extract was determined.[4] Then, raw protein content of the algal samples was estimated by optimizing the extraction of the protein fraction from the microalgae matrix, carrying out a Vis-spectrophotometric determination. Furthermore, thanks to a basic protein hydrolysis, a new GC-MS method was validated for the evaluation of the amino acidic content including tryptophan. In addition, since microalgae can be contaminated by bisphenol A (BPA), that is responsible for many toxic effects on humans, its content in microalgae as contaminant was verified according to the European legislation limit of 50 µg kg^{-1} of food weight.[5,6] BPA was derivatized by using BSTFA-TMCS and it was analysed by GC-MS in SIM mode. A DOE optimization study of the reaction conditions was performed. In particular, the proposed method can be considered for the determination of BPA in microalgal dried biomass samples used for food supplements or cosmetic products and it can support the analysis in the microalgae industries as quality control microalgae processing thus minimizing the impact on the environment. Finally, considering all the obtained data, a principal component analysis (PCA) plots was performed and the two microalgal species were clustered in terms of their micro and macro nutrients. That allowed to differentiate their properties and to emphasize their differences in content and activity. In conclusion, specific claims have been identified for each class, which allow them to be addressed to defined end users in view of their potential usage in cosmetics, food supplements and as food.

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OC19

CHEMICAL CHARACTERIZATION AND VALORIZATION OF *H. italicum* AND MEDICINAL MUSHROOMS FOR NUTRACEUTICAL APPLICATIONS

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The present study is part of the wider "Valpharmarecchia" project, funded by the Emilia Romagna Region,[1] which aims to develop new nutraceutical and cosmeceutical, based on medicinal mushrooms and medicinal herbs from Marecchia Valley.Medicinal mushrooms are known for their low fat content, high fiber content, triterpenes, phenolic compounds, sterols, proteins and polysaccharides such as β-glucans.[2] Several researches have shown the benefits of these polysaccharides, acting favorably on the gut microbiota and immune system.[3] Consequently, we focused on the development of a dietary supplement based on ascorbic acid, *Helichrysum italicum* extracts and *Pleurotus eryngii*, which due to its high intake of β-glucans would contribute to the normal function of the immune system and the protection of cells from oxidative stress.Among the various plants growing wild in the Marecchia area, *H. italicum* was chosen because of its potential as a possible source of biologically active molecules. The phytochemical profile of *Helichrysum* is characterized by several classes of compounds, the most common of which include terpenes and phenolic compounds.[4] In this work, several extracts were characterized for polyphenol content by Folin-Ciocâlteau assay and for antioxidant activity by PCL, FRAP and DPPH analysis.

Moreover, in order to make unique extracts, we applied convergent analytical methods, such as XRF, EA-IRMS, ICP-QQQ-MS and genetic analysis, achieving a further objective of the study, namely the enhancement of the agronomic heritage of the Upper Marecchia Valley. In this regard, geochemical and isotopic characterization of soils and flowering tops of *H. italicum* from seven localities within the territory of the Valmarecchia was conducted, and the genetic variability of twelve *Helichrysum* samples from different origins was evaluated using ISSR (Inter Simple Sequence Repeats) markers, with the aim of deepening knowledge and differences between native and commercial *Helichrysum*.

The project has the ambition of becoming a useful model to be extended nationwide, thus contributing to the development of circular economy approaches.

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OC20

A PERSONALIZED URINARY METABOLIC PROFILE FOR BLADDER CANCER PATIENTS: AN APPLICATION OF THE SYNHMET METHOD

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Bladder cancer (BC) is the most common cancer of the urinary tract1 and a leading cause of mortality worldwide, with approximately 550,000 new cases and 160,000 deaths per year2. BC tumors are divided into two classes depending on whether they invade the detrusor muscle (muscle-invasive bladder cancer, MIBC) or not (non-muscle-invasive bladder cancer, NMIBC). The first presents a higher risk of metastasis of lymph nodes or other organs but, fortunately, represents only 25% of diagnosticated BC cases3. Evaluating patients suspected of having BC is performed using cystoscopy, an invasive method currently considered the gold standard. Using urine as a biofluid for diagnosis or prognosis of any disease has always been considered the most advantageous, mainly because of the easy and rapid collection and non-invasiveness. No urinary-based tumor markers have demonstrated sufficient sensitivity and specificity to replace cystoscopy in detecting BC4–6. In this work, we measured 165 metabolite concentrations in urine from a group of bladder cancer patients and controls, obtaining in this way the most extensive quantitative urinary profile known so far. The method that enabled such a complete result is based on the synergistic use of the two leading techniques in metabolomics: Nuclear Magnetic Resonance (NMR) spectroscopy and Ultra-High Pressure Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UHPLC-HRMS). A recently developed protocol, SYHNMET7, was applied for this purpose. With this approach, orthogonal information such as the chemical shift from NMR, the retention time, and the accurate mass from UHPLC-HRMS are correlated to identify and accurately quantify endogenous urinary metabolites. This method made it possible to extract crucial information to investigate how the body's systemic response to the presence of the tumor. The results from this analysis were then correlated with those obtained from twenty-five metabolomics papers published in the search for non-invasive urinary diagnostic biomarkers8. The outcome of this analysis opens up scenarios for debate on how much systemic response has to offer in the presence of a disease such as BC.

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OC21

1,4,5-TRISUBSTITUTED 1,2,3-TRIAZOLE DERIVATIVES AS HSP90 INHIBITORS: SYNTHESIS, EVALUATION, AND STRUCTURAL CHARACTERIZATION

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Heat shock protein 90 (Hsp90) is a ubiquitous molecular chaperone that stabilizes client proteins in a folded and functional state, using ATP hydrolysis as a source of energy. Hsp90 is composed of two identical and symmetrical subunits and each monomer consists of three domains, the N-terminal (NTD), the middle (MD), and the C-terminal domain (CTD). The NTD contains the main structural elements generating the ATP binding site in which the ATP substrate is hydrolyzed. Molecules preventing ATP hydrolysis act as Hsp90 inhibitors, blocking its chaperone activity, and subsequently leading to client protein degradation and cell death.[1] Therefore, human Hsp90 represents a validated target for developing new anticancer agents. To this aim, Ruthenium catalysed azide-alkyne cycloaddition (RuAAC) reactions have been used to generate a new family of 1,4,5-trisubstituted triazole carboxylic acid derivatives that showed high affinity toward Hsp90.[2] Differently form Cu(I)-catalysed reactions, RuAAC reactions works with both terminal and internal alkynes and in fact, this represents the preferred way to synthesize 1,4,5-trisubstituted-1,2,3-triazoles. In these molecules, the concomitant presence of a resorcinol-like moiety, an aryl group, and an alkyl amide in position 4 of the triazole ring represent essential features accounting for their potent inhibitory activity. The most promising inhibitors of the series showed Hsp90 binding in the single digit nanomolar concentration and displayed antiproliferative activity toward non-small cell lung carcinoma NCI-H460 and remarkable *in vivo* anticancer activity. In addition, the structural characterization of the human Hsp90-NTD in complex with one of these inhibitors has been performed through X-ray crystallography. The structure has shown significant conformational changes in the area surrounding the catalytic site, to which the compound is bound, in comparison to ligand-free Hsp90-NTD and its complexes with ATP and ADP analogues.[3] The structural information obtained has allowed to evaluate the key structural determinants responsible for inhibitors binding.

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OC22

PROGRESS ON POTENT AND SPECIFIC PARP INHIBITORS: A SAR STUDY AROUND THE [1,2,4]TRIAZOLO[3,4-*b*]BENZOTHIAZOLE SCAFFOLD

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The PARPs family accounts for 17 enzymes that are able to catalyze the covalent attachment of ADP-ribose derived from NAD^+ to a target macromolecule as reversible post-translational modification. The ADP-ribose can be attached as a monomer (MARylation) or linear and branched polymers (PARylation). For the essential roles played by PARylating enzymes, they have been already validated as drug targets,[1] with four drugs targeting PARP1/2 approved since 2014 as anticancer precision medicines.

On the contrary, MARylating enzymes remained understudied for a long time. However, recent studies evidenced their key roles both in physio- and pathological processes underlining the possibility to also exploit them as drug targets.[2] This was recently further confirmed by two inhibitors that entered clinical trials.

Nonetheless, specific inhibitors are urgently needed to better understand the roles of these less explored enzymes and to identify new clinical candidates.

In this context, we have recently identified a new chemical entity, the [1,2,4]triazolo[3,4-*b*]benzothiazole (TBT) scaffold, that, based on the substitution pattern, is able discriminate the various PARPs subfamilies and specifically inhibit them.[3] Starting from the lead compound, which represents the most potent PARP10, PARP12 and PARP15 inhibitor ever reported to date, we continued to work around the TBT nucleus.

In particular, new derivatives have been designed by following the information derived from the co-crystal structures in order to improve specificity and potency by reaching less conserved regions of the PARPs catalytic domain. The new designed derivatives have been then synthesized and profiled for both biological and ADME-tox properties, with some of them that emerged as particularly interesting. In addition, some structural requirements emerged as precious to achieve specificity and better delineate the TBT SAR.

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OC24

INDOLYL DIKETO ACID DERIVATIVES AS INHIBITORS OF NSP13 OF SARS-COV-2 THAT BLOCK VIRAL REPLICATION

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The SARS-CoV-2 non-structural protein 13 (nsp13) helicase is an essential enzyme for viral replication. The CoVs nsp13 is a multidomain enzyme that can unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. Nsp13 is a highly conserved protein among all known coronaviruses, and, is one of the most explored viral targets to identify new possible antiviral agents.[1] Following the first report on DKAs as micromolar inhibitors of the unwinding of SARS-CoV-1 nsp13 we explored SARS-CoV-2 nsp13 as target for our in-house library of DKAs. Two indolyl DKA compounds[2] emerged as promising hits, being able to block both nsp13 catalytic activities in the low micromolar range. We designed and synthesized a small set of analogues, characterized by an indolyl core endowed with a diketohexenoic chain in 3-position, to further explore the SARS-CoV-2 nsp13 inhibition and the antiviral activities. All tested compounds were able to block both nsp13 enzymatic activities in the low micromolar range. Mode-of-action studies revealed ATP-non-competitive kinetics of inhibition, not affected by substrate-displacement effect, suggesting an allosteric binding that was further supported by molecular modelling calculations. Moreover, four compounds, were able to inhibit viral replication in the low micromolar/submicromolar range without exerting cytotoxicity at the tested concentration.

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OC25

VALIDATION OF A NEW LC-HRMS METHOD FOR THE QUANTIFICATION OF THE CHEMICAL CHAPERONE 4-PHENYLBUTYRIC ACID (4-PBA) IN CELL CULTURE MEDIA

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Chemical chaperones are small molecules which nonspecifically interact with proteins and generate a solvophobic force capable of preventing denaturation and promoting protein trafficking improvements.[1-2] In recent years, 4-phenylbutyric acid (4-PBA), an FDA-approved drug, has increasingly been used as a nonspecific chemical chaperone *in vitro* and *in vitro*, but its pharmacodynamics is still not clear.[2-5] Many *in vitro* studies are arising with the aim of identifying the mechanism of action of 4-PBA, and to describe the biomodulation driven by this drug, or using 4-PBA as an ER stress and protein synthesis modulator; different biological readouts have been used to describe the 4-PBA effects. In this context, a method to assess 4-PBA absorption in different cell types would be useful to better define the treatment concentration, and assess the implication of co-treatment with other drugs. In light of a study reported in literature[2] and the increasing interest in cell culture media, including whole secretome, extracellular vesicles, and disease treatment, it might be of paramount importance, for the correct design of experiments, to know the stability and absorption of 4-PBA. To the best of our knowledge, the determination and quantification of 4-PBA in cell culture media have not been particularly dealt with in bioanalytical research even though several studies have reported the effect and concentration of 4-PBA and its metabolites in biological matrices, such as plasma and urine, using liquid and gas chromatography methods coupled with diode array detectors (DAD) or mass spectrometry (MS). However, none of these methods was developed to quantify 4-PBA in cell culture media. In this context, in vitro assessment of 4-PBA extracellular concentration could be a new strategy to underpin many pharmacological approaches, widely spread in the literature which have been comparing its biological effects to the surrounding cellular microenvironment. In this investigation, we describe the development and validation of a new LC-HRMS method to quantify 4-PBA, but also to monitor possible combined treatment, in order to investigate its in vitro behavior and effects. The validation was successfully performed in Dulbecco's Modified Eagle Medium (DMEM), one of the most common medium for cell culture and Neurobasal A medium (NBA), a specific medium for CNS (Central Nervous System) cells' support.

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OC26

FROM 2,1-BENZOTHIAZINE 2,2-DIOXIDE DERIVATIVES TO SULFONYL ANTHRANILIC ACID ANALGOUES AS POTENT PAN SEROTYPE DENGUE INHIBITORS

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Dengue virus (DENV), consisting of 4 serotypes (DENV-1-4), is a (+)-single-stranded RNA virus belonging to the flavivirus genus and represents a major public health threat in many countries with no approved antiviral therapeutics available.[1] In this work, we designed and synthesized new sulfonyl anthranilic acid (SAA) derivatives by exploiting a scaffold morphing approach of the 2,1-benzothiazine 2,2-dioxide core, previously used by us to develop DENV polymerase inhibitors that resulted inactive in cell context (derivative **1** in Figure 1).[2] New SAA derivatives gained cell-based anti-DENV-2 activity, while losing the ability to inhibit DENV-2 polymerase. Best analogue **2** (Figure 1) showed an EC_{50} of 0.87 µM against DENV-2 with a CC_{50} >100 µM on Huh-7 cells and exhibited a DENV pan-serotype activity.[3] Additional studies on compound **2** confirmed the lack of DENV-2 polymerase and protease inhibition and highlighted an antiviral activity at a post-entry step, likely involving a host target. Indeed, derivative **2** exhibited anti-DENV-2 activity only in different human cell lines, while resulting inactive on mosquito and hamster cells. Further efforts aimed at: i) identifying additional SAA derivatives with improved pharmacokinetic properties and ii) isolating the molecular target are ongoing and will be discussed in this work.

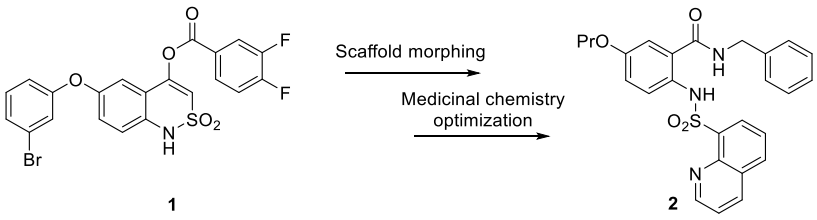

Figure 1. From 2,1-benzothiazine 2,2-dioxide scaffold to sulfonyl anthranilic acid derivatives.

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OC27

OC QM/MM MODELLING OF MGL CARBAMOYLATION BY PIPERAZINE AZOLE UREAS REVEALS THE ROLE OF LEAVING GROUP EXPULSION AND DISCRIMINATES INHIBITORS WITH HIGH AND LOW POTENCY

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Monoglyceride lipase (MGL) is a serine hydrolase that catalyzes the deactivating cleavage of the endocannabinoid 2-arachidonoylglycerol (2-AG), which exerts neuroprotective effects.[1] MGL inhibition prevents 2-AG hydrolysis and has emerged as a promising strategy for treating neuroinflammatory diseases.[1] Piperazine azole ureas represent an effective class of MGL inhibitors and are able to covalently modify the catalytic nucleophile Ser122.[2] To get mechanistic insights, useful to rationalize structure-activity relationship (SAR) data, we investigated the mechanism of MGL carbamoylation by the reference triazole urea SAR629 (IC_{50} = 0.2 nM)[2] and two recently described inhibitors featuring a pyrazole (IC_{50} =1800 nM) or a 4-cyanopyrazole (IC_{50} = 8 nM) leaving group,[3] using a hybrid quantum mechanics/molecular mechanics (QM/MM) approach. Simulations indicated that changes in the electronic structure of the leaving group strongly affect reaction energetics, with triazole and 4-cyanopyrazole inhibitors following a more accessible reaction path compared to the pyrazole derivative.[4] The protocol provided reaction barriers able to discriminate between MGL inhibitors with different potencies and resulted able to correctly predict the activity of a pyrazole-4-carboxamide urea recently reported in a granted patent ($IC_{50} \geq 100$ nM).[4,5] These results highlight the ability of our QM/MM approach to elucidate SAR data and provide insights for the design of novel MGL inhibitors.[4]

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OC28

TETRAZOLE AND OXADIAZOLE DERIVATIVES OF TARIQUIDAR AS POTENT MDR REVERSERS

Braconi, L.;[a] Dei, S.;[a] Contino, M.;[b] Riganti, C.;[c] Bartolucci, G.;[a] Manetti, D.;[a] Romanelli, M.N.;[a] Perrone, M.G.;[b] Colabufo, N.A.;[b] Guglielmo, S.[d] and Teodori E.[a]

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In many resistant tumors, multidrug resistance (MDR) is related to an overexpression of efflux pumps, as P-gp, MRP1 and BCRP, which actively reduced the intracellular concentration of anticancer drugs, leading to chemotherapy treatment failure.[1] A strategy to overcome MDR is the co-administration of an inhibitor of these proteins with anticancer drugs, to restore their cytotoxic effect in resistant tumor cells.[2] To investigate the SARs of tariquidar, a well-known P-gp inhibitor, we replaced its amide group with two bioisosteric functions, the tetrazole and oxadiazole rings, synthesizing new MDR reversers (Figure 1). In these new compounds, the heterocycles were linked to methoxy-substituted aryl moieties, already present in potent P-gp modulators.[3]

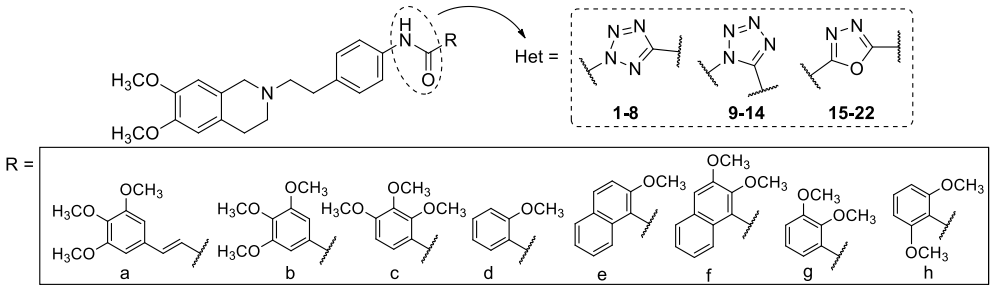

Figure 1. New tetrazole and oxadiazole derivatives of tariquidar.

Several biological tests and an *in silico* study were performed on these new compounds. Results showed that all derivatives inhibited P-gp with high potency, reducing the P-gp-mediated efflux of a fluorescent probe in MDCK-MDR1 cells: in this assay, two compounds reached EC_{50} values in the low nanomolar range, showing higher activity than tariquidar. Interestingly, an oxadiazole derivative emerged as a dual P-gp/BCRP inhibitor. Moreover, many compounds enhanced the antiproliferative effect of the P-gp substrate doxorubicin in cells overexpressing P-gp (MDCK-MDR1 and HT29/DX cells). Indeed, they increased the intracellular accumulation of doxorubicin, through the inhibition of P-gp efflux activity. All these results confirmed that our new tetrazole and oxadiazole derivatives are potent MDR reversers.

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OC29

A NEW ANTIDIABETIC AGENT SHOWING PPARα/γ DUAL AGONISM AND MITOCHONDRIAL PYRUVATE CARRIER INHIBITION

Cerchia, C.;[a] Laghezza, A.;[b] Genovese, M.;[c] Leuci, R.;[b] Pranzini, E.;[c] Santi, A.;[c] Brunetti, L.;[b] Piemontese, L.;[b] Tortorella, P.;[b] Biswas, A.;[d] Singh, R. P.;[d] Tambe, S.;[e] CA, S.;[f] Pattnaik, A. K.;[d] Jayaprakash, V.;[d] Paoli, P.;[c] Loiodice, F.[b] and Lavecchia, A.[a]

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that play a key role in the regulation of a large number of genes whose products are directly or indirectly involved in glucose homeostasis and lipid metabolism.[1] Therefore, they have been considered suitable targets for the treatment of metabolic disorders. The PPAR family comprises three different subtypes: α, β/δ, and γ, whose expression and actions differ according to subtype, organ, and tissue cell type. A new series of derivatives of a previously reported PPARα/γ dual agonist, LT175,[2] allowed the identification of compound **10**.[3] This latter was able to potently activate both PPARα and -γ subtypes as full and partial agonist, respectively. Computational studies were performed to provide a molecular explanation for this different behaviour on the two different targets. In vivo experiments showed that this compound induced a significant reduction in blood glucose and lipid levels in an STZ-induced diabetic mouse model, while displaying no toxic effects on bone, kidney, and liver. By examining the antihyperglycemic activity of **10**, we found out that it produced a slight but significant inhibition of the mitochondrial pyruvate carrier, acting also through insulin-independent mechanisms. This is the first example of a PPARα/γ dual agonist reported to show this inhibitory effect, thus representing the potential lead of a new class of drugs for treatment of dyslipidemic type 2 diabetes.[3]

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OC30

ENSEMBLE OF STRUCTURE AND LIGAND-BASED CLASSIFICATION MODELS FOR hERG LIABILITY PROFILING

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Cardiotoxicity represents one of the most frequent adverse registered during clinical studies and post-approval surveillance. Drug-induced cardiotoxicity is often related to the blockade of the human ether-à-go-go-related potassium channel (hERG) causing the prolongation of the cardiac repolarization which might result in fatal ventricular arrhythmias. Therefore, assessing the hERG-safety profile of new drug candidates in the early stages of drug discovery is crucial to prevent undesired cardiotoxic effects. In this context, the advancement of machine learning (ML) algorithms in drug discovery programs enabled the development of predictive models able to identify potential hERG binders mainly basing on ligand-based (LB) descriptors.[1] However, LB models present a limited applicability domain (AD) and often fail in predicting new chemotypes different to those used for the model generation. Recently, the experimental structure of hERG channel in complex with the inhibitor astemizole (Figure 1) has been solved[2] paving the way towards the use of structure-based (SB) approaches allowing to overcome the limitations of LB methods. In this work, both LB and SB classifiers for hERG-related cardiotoxicity were built by means of Random Forest algorithm, employing a training set composed of 12789 hERG binders. In more details, the SB models were trained on a set of scoring functions generated by docking and rescoring calculations while the LB classifier was built on a set of physicochemical descriptors and fingerprints. All the generated models were subjected to an internal validation, by ten-fold cross-validation, and further verified on an external test set. Models combining LB and SB features were developed as well. The outcomes revealed that the LB model outperform the SB models in cross-validation experiments, while the model integrating LB and SB features showed the best performance when applied to the external test set, highlighting the benefits of integrating LB and SB attributes in correctly classifying unseen molecules. AD evaluation was carried out basing on four different methods revealing the wider AD of SB models compared to the LB classifier. Finally, the comparison with other hERG models published in literature was performed.[3]

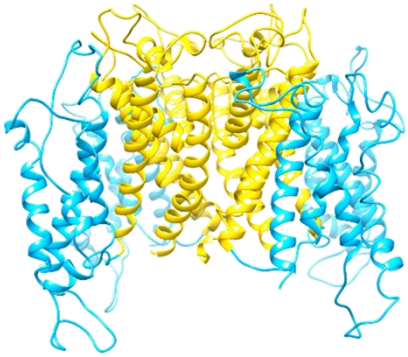

Figure 1. X-ray structure of hERG channel in complex with the inhibitor astemizole (PDB ID 7CN1)

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OC31

SYNTHESIS, COMPUTATIONAL INSIGHTS, AND EVALUATION OF NOVEL SIGMA RECEPTORS LIGANDS

Dichiara, M.;[a] Ambrosio, F. A.;[b] Barbaraci, C.;[a] Shah, D.;[c] González-Cano, R.;[d] Ruiz-Cantero, C.;[d] Coricello, A.;[e] Costa, G.;[e,f] Lee, S. M.;[c] Son, K. N.;[c] Lombino, J.;[a] Costanzo, G.;[a] Cosentino, G.;[a] Giordano, G.;[a] Pasquinucci, L.;[a] Marrazzo, A.;[a] Aakalu, V. K.;[c] Cobos, E. J.;[d] Alcaro, S.;[e,f] and Amata, E.[a]

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Over the years, spirocyclic and fused scaffolds have gained increasing interest in the development of bioactive compounds due to their peculiar spatial arrangement affecting crucial parameters of drug candidates, including potency, selectivity, and physicochemical properties.[1]

With the aim of identifying classes of Sigma Receptors (SRs) ligands with high affinity and selectivity, novel SR ligands – based on diazaspiro, diazabiciclo, dihydropyrrolopyrazole, as well as other scaffolds – have been developed. By varying the central cores, and the substituents decorating them, we were able to capture discrepancy among S1R *vs* S2R affinity and functional profile, as well as the behavior in a model of in vivo disease (Figure 1).

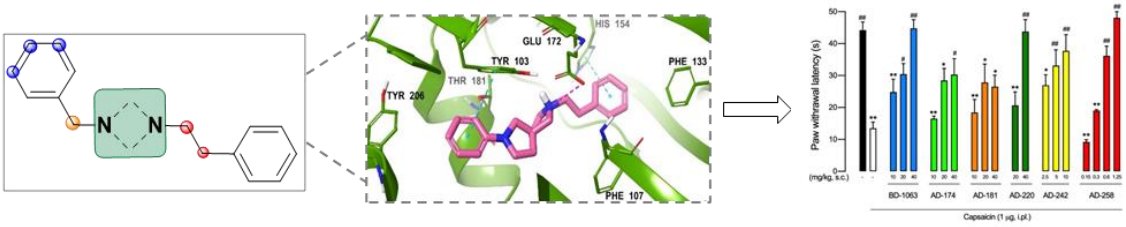

Figure 1. General workflow in the development of novel SR ligands.

Our efforts have been aimed at developing new candidates based on affinity and selectivity and on the synthesis of new compounds for additional rounds of drug-target evaluation. Molecular modeling studies were carried out to analyze the binding mode and the interactions established between the ligands and S1R and S2R. The most notable compounds have been subjected to further biological evaluation in vitro and in vivo models.

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OC32

2-DIFLUOROMETHYL-1,3,4-OXADIAZOLE – TOWARDS ABSOLUTE SELECTIVITY VS HDAC6

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HDAC6 primarily localizes in the cytoplasm and mainly deacetylates cytoplasmic proteins, contributing to cellular homeostasis at multiple levels. Dysregulation of its activity is linked to the development of diseases. These factors, combined with the safety of HDAC6 inhibitors, have established HDAC6 as an intriguing pharmacological target. Particularly, substantial evidence suggests the involvement of HDAC6 in chemotherapy-induced peripheral neuropathy (CIPN), a side effect, which is common among various chemotherapeutics and currently lacks an available cure. Here, we present a novel class of potent and highly selective non-hydroxamate HDAC6 inhibitors featuring a 2-difluoromethyl-1,3,4-oxadiazole (DFMO) group as a warhead.[1] This moiety exhibits a distinctive mechanism of action (fig. 1),[2] potentially accounting for its remarkable selectivity towards HDAC6. In this novel category, we have identified compounds that possess outstanding inhibitory properties along with favorable pharmacokinetic profiles. The most promising molecules have exhibited efficacy in both preventive and curative settings of *in vitro* and *in vivo* models for CIPN.

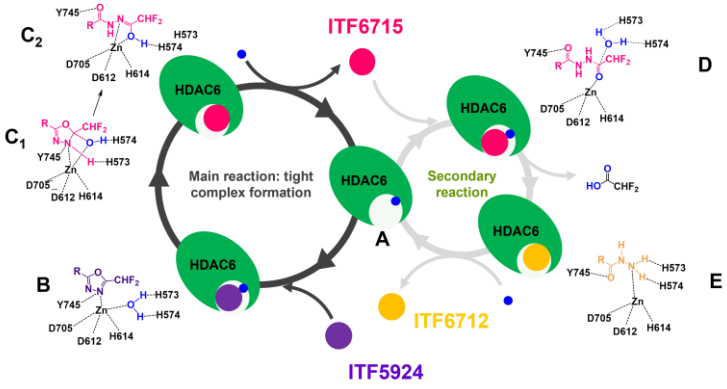

Figure 1. The proposed mechanism of inhibition for DFMO-based inhibitors**.**

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OC33

THE ROLE OF HYDROPHOBICITY IN DRUG DISCOVERY: FROM 3D-QSAR MODELS TO HUGE CHEMICAL LIBRARIES EXPLORATION

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Solvation is a crucial factor in determining the recognition and binding affinity between small ligands and their target receptors. The chemical structure of a drug-like candidate must encode an appropriate balance between hydrophilic and hydrophobic groups in order to sustain an adequate pharmacokinetic profile and to modulate the energy penalty due to desolvation upon ligand binding. Furthermore, the three-dimensional (3D) distribution of polar/apolar groups in the bioactive species must be optimal to guarantee the hydrophobic complementarity with the residues that shape the binding pocket.

In this talk, we will present the application of quantum mechanically derived hydrophobic descriptors in the discovery of novel sEH inhibitors which play a role in several diseases, including hypertension, cardiac hypertrophy, arteriosclerosis. Three-dimensional quantitative structure–activity relationship (3D-QSAR) pharmacophores were generated by combining hydrophobic and hydrogen-bond parameters in conjunction with a tailored list of 76 known sEH inhibitors. Then, a prospective study was performed including a virtual screening of two chemical libraries to identify new potential hits, which were subsequently experimentally tested for their inhibitory activity on human, rat, and mouse sEH. This led to the identification of six inhibitors with IC50 < 20 nM, including two with IC50 values of 0.4 and 0.7 nM. [1]

Moreover, we will present how the same 3D hydrophobic molecular descriptors are being applied in virtual screening using commercially available libraries (in the order of few million compounds) and ultra-large chemical libraries such as EnamineREAL (31 billion compounds). In particular, we will show the value of hydrophobic descriptors in virtual screenings for several receptors giving special attention to the chemical diversity of the retrieved structures.

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OC34

LEAD OPTIMIZATION OF HUMAN THYMIDYLATE SYNTHASE DIMER DISRUPTERS: FROM COMPUTATIONAL STUDIES TO EVALUATION OF THEIR BIOLOGICAL PROFILES

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Human thymidylate synthase is a homodimer of 76 kDa with two active sites formed by residues of both the monomers. This protein is involved in the conversion of dUMP to dTMP through a reductive methylation of the co-factor mTHF.[1] Due to hTS role, this enzyme has been used as target in cancer. Drugs like 5-fluorouracil (5-FU), Decitarbine and folates analogues like Raltitrexed are able to covalently bind the active site of the enzyme Due to their mechanism, there is an overexpression of the enzyme inside the cell leading to drug resistance. During the years, evidence have proven that hTS dimer is in equilibrium with its inactive monomers.[2] To overcome the drug-resistance, our research group has aimed to find new ways to interfere with this equilibrium to inactivate the enzyme without causing its overexpression by targeting the interface of the dimer shifting the equilibrium toward the monomers.[3] From a library of more than 150 compounds developed as new hTS inhibitors two molecules have been selected as lead for the optimization studies: E7 and AIC-A16. Aim of this optimization was increase the solubility, replacement of the toxicophore nitro (NO2) with others EWG group like CF3, increased activity on the recombinant protein and on cells.

Our optimization started with the analysis of the interactions between the leads and the proteins. To perform the computational studies, we used Maestro to perform docking simulations (Glide) and Induced-fit dockings (Glide+ Prime). We docked our leads in the peptide site of the x-ray crystal structure (3N5E). From the data obtained we designed a library of 22 new dimer disrupters that gave during simulation good to optimal scores >7.000 kcal/mol. The selected compounds have been synthetized, purified (HPLC purity >99%) and characterized with good yields (20-95%). All compounds have been tested spectrophotometrically against hTS to evaluate the Ki (Ki 1-10 µM) on the recombinant protein and the effects of compounds on cell growth has been determined by the crystal violet dye assay on HT29 and HCT116 cell lines of colorectal cancer.

The results obtained by this optimization process have shown that we successfully increased the activity on cells compared to the lead AIC-A16 in some cases to 60 % of growth inhibition. We have successfully removed the nitro group leading to more active compounds than the lead. In conclusion, we succeed in the optimization of the selected lead.

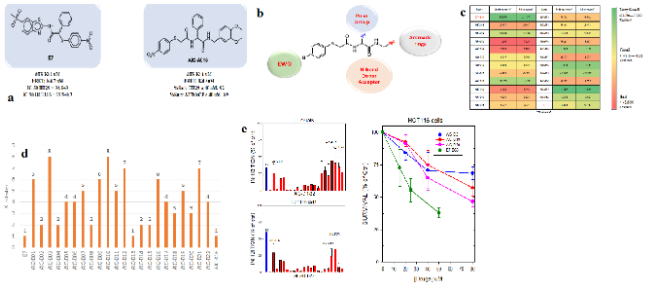

Figure 1, a. Structure and biological profiles of Leads. b. Site of modifications. c. Docking score heat map. d. Ki values for AIC-D. e. growth inhibition % on HT29 and HCT 116.

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FLASH communications

BEST DOCTORAL THESIS AWARDS

DEVELOPMENT OF SUSTAINABLE AND EFFICIENT TAILOR-MADE PROCESSES FOR PHARMA AND FOOD APPLICATIONS

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The combination of two enabling technologies, such as flow chemistry and biocatalysis, is a key strategy for the development of sustainable and scalable processes to obtain biologically active compounds for food and pharma applications, minimizing the ecological burden. This strategy allows to exploit the remarkable advantages associated with the continuous synthesis, *i.e.*, better mass transfer, temperature control and mixing efficiency, the possibility to insert in-line analysis and purification steps, in combination with the mild operational conditions required by biocatalysts together with their high chemo- regio- and stereo-selectivity.[1]

The main focus of this project was to display how versatile and tunable the combination of biocatalysis and flow chemistry could be for the synthesis of different biologically active compounds, improving established classic but polluting chemical approaches or clearing the way for larger scale manufacturing. Three main biocatalyzed reactions that have been explored: i) reactions of functional group transfer (from one molecule to another), ii) redox reactions and iii) condensation reactions for the synthesis of different functional groups (i.e., esters, carbonate, carbamate). In some cases, a combination of more than one biocatalyst was used in the same protocol to perform further modifications of the high value chemicals obtained. To further improve the environmental benignity of the procedures, when possible, classical organic solvents were replaced by greener options, i.e., solvents derived from renewable sources and new solvent systems like natural deep eutectic solvents (NADESs). Moreover, in-line purification procedures have been designed to obtain the desired compounds without the need of additional purification steps.[2-5]

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DEVELOPMENT OF THERAPEUTIC AND DIAGNOSTIC AGENTS TARGETING TUMOR LIPID AND SUGAR METABOLISM

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Cancer cells exhibit an uncontrolled and rapid growth due to malfunctions in the regulatory system governing cells' proliferation and division. This unrestrained proliferation of cancer cells necessitates a huge amount of nutrients and energy, leading to alterations and adaptations in their metabolic profile. Indeed, the metabolic reprogramming becomes essential to satisfy the rapid growth of the tumor and to ensure its survival under adverse conditions. Notably, cancer cells display dysregulation in carbohydrate, amino acid (particularly glutamine), and lipid metabolism. Therefore, the goal of my PhD Thesis was to develop therapeutic and diagnostic agents able to interfere with tumor lipid and sugar metabolism. Regarding the reprogrammed lipid metabolism, I focused on one key enzyme: monoacylglycerol lipase (MAGL), which plays a central role in cancer progression, invasiveness and aggressiveness. Specifically, the aim was to design and synthesize small organic compounds able to reversibly inhibit MAGL with potential anti-cancer activity.[1,2] Another goal of my Thesis was the development of the first PROteolysis Targeting Chimera (PROTAC) small molecules targeting MAGL (anti-MAGL PROTACs). These molecules were designed to induce ubiquitination of the enzyme, leading to its subsequent degradation. For what concern altered glucose metabolism, the final objective of my Thesis was to develop glycoconjugated metal complexes as diagnostic probes for the selective IR visualization of glycolytic cancer cells by using the well-known metabolic switch of tumors called Warburg effect.

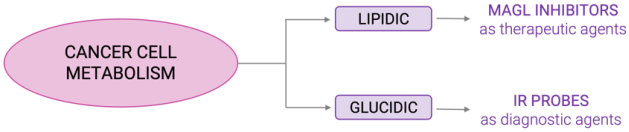

Figure 1. Schematic representation of my PhD Thesis.

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FC1

EXPLOITING THE FEATURES OF SHORT PEPTIDES TO RECOGNIZE SPECIFIC CELL SURFACE MARKERS

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Stem cells have an unlimited potential for cell division and the ability to transdifferentiate into other cell types and in recent years they grew to be a first-line weapon of regenerative medicine for repairing tissue and organic abnormalities caused by diseases, congenital deficiencies, and age-related effects[1]. Bone marrow-derived mesenchymal stem cells (MSCs) mainly develop in adipocytes, chondroblasts, and osteoblasts but also show the property to successfully transdifferentiate in neural, myocyte, and epidermal cells when engrafted in endogenous tissues under specific conditions[2,3]. Efficient isolation of MSCs from other cell components in the bone marrow is fundamental to obtaining valuable MSC clinical applications[4]. Currently, the most widespread purification methods include the isolation of MSCs based on the expression of precise surface markers, thus involving specific antibodies[5-7]. In this work, we aimed to develop a fast and affordable method for the phenotypic characterization and isolation of MSCs from bone marrow without using immunoglobulins. To this purpose, we designed short peptides based on the deposited 3D structures of two specific MSCs surface markers, CD44 and CD271, in complex with their natural ligands. The peptides were selected by computational modeling, synthesized by solid-phase peptide synthesis, and tested in cell assays. Interestingly the data show that starting from a small set of peptides, using a protocol based on molecular simulations, it is possible to obtain small ligands endowed with a significant specificity in targeting CD44 and CD271 in place of their antibody, bypassing the common challenges and issues due to the use of immunoglobulins.

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FC2

TARGETING ROS PRODUCTION THROUGH INHIBITION OF NADPH OXIDASE

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NADPH oxidases (NOXs) are enzymes responsible for only generating reactive oxygen species (ROS)[1]. The seven NOX isoforms participate in various physiological processes, including cell proliferation[2], and their deregulation has been associated with various cancers, such as colon cancer[3] and leukaemia[4]. In order to identify *bona-fide* NOXs inhibitors, we firstly complicated the structure of the covalent NOX inhibitor **VAS2870** (see **Figure 1**). We observed that replacing of the 2-thiobenzoxazole leaving group of **VAS2870** with different functional groups abolished NOXs inhibition. By inserting in the para position of the benzyl group of **VAS2870** the pharmacophore moieties able to create a covalent FAD isoalloxazine-ligand adduct, we obtain **MC4762** and **MC4767** that are not able to provide an adduct with FAD but **MC4762** selectively inhibits NOX2 while **MC4767**, and especially its satured analogue **MC4768**, increased the *Cylindrospermum stagnale* (*cs*) NOX5 DH domain denaturation temperature (ΔT_m). To identify *bona-fide* NOXs inhibitors, we also simplified the molecular structure of the lead compound **M41** that came out from an *in silico* screening (see **Figure 1**). We observed that the opening of the central ring (**MC4793**) abolished human NOXs inhibition. Furtherly, we obtained **MC4876** that has a significant selectivity towards *h*NOX2 and has micromolar EC_{50} values on NOX2, NOX4, and NOX5 expressing cells. Moreover, we observed that differentiation of monocytic U937 cells into macrophages does not greatly alter the sensitivity to **MC4876** (same result for **M41**). Notably, its synthetic intermediate **MC4854** is a powerful (sub-micromolar) and selective inhibitor of *h*NOX5 and it also resulted a good inhibitor against cellular NOX5 with 79% inhibition. These results confirm the feasibility of achieving isoenzyme-specific NOX inhibitors by modifying the **M41**.[5]

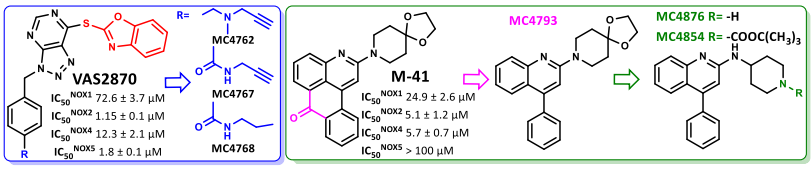

Figure 1.

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FC3

STRUCTURE-BASED DRUG DESIGN OF NEW SIGMA1 AGONISTS FOR HUNTINGTON'S DISEASE TREATMENT

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Huntington's disease (HD) is an autosomal dominant disorder caused by a mutation in the HTT gene, which progressively leads neurons in parts of the brain to break down and die. Unfortunately, to date there are no effective treatments that can stop or prevent the onset of this devastating disease. However, recent and growing numbers of studies are showing how the sigma-1 receptor (σ1R) may be implicated in the control of several neurodegenerative disorders, including HD1. The σ1R is a small and poorly understood membrane receptor expressed in the central nervous system, whose 3D structure has been recently determined by X-ray crystallography and responding to different synthetic ligands such as (+)-pentazocine (agonist) and haloperidol (antagonist). Substantial evidence suggests that knockdown or antagonism of the σ1R has analgesic effects, while agonists have been shown to have neuroprotective activity in neurodegenerative diseases. Nevertheless, the structural basis for agonism or antagonism on σ1R is largely unknown. In general, the overall conformation of the receptor bound to the agonist crystallizes similarly to that bound to the antagonist, except for a shift of about 1.8Å in the α4 helix2. Probably, this shift is responsible for the tendency of agonists to decrease the oligomeric state of the protein. Through structure-based computational methods, we aim to design new small molecules as σ1R modulators. Indeed, very recently, a high binding affinity for the σ1R of the antipsychotic Iloperidone has been demonstrated3. From our early studies, the pharmacophoric groups have emerged. In detail, the most stable interactions are established by the nitrogen atom of the piperidine ring of Iloperidone, which is positively charged at physiological pH. This charge allows the molecule to interact with the Phe107 of protein and the negatively charged Glu172 residue. Starting to these data, the chemical structure of this antipsychotic drug will be modified applying a scaffold hopping approach, in order to obtain a pronounced and selective agonist of the σ1R.

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FC4

TARGETING THE CONSERVED ACTIVE SITE OF SPLICING MACHINES WITH SPECIFIC AND SELECTIVE SMALL MOLECULE MODULATORS

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The self-splicing group II introns are bacterial and organellar ancestors of the nuclear spliceosome and retro-transposable elements of pharmacological and biotechnological importance[1]. Integrating enzymatic, crystallographic, and simulation[2,3] studies, we demonstrate how these introns recognize small molecules through their conserved active site. These RNA binding small molecules selectively inhibit the two steps of splicing by competing with the substrates of the splicing reaction and by preventing crucial active site conformational changes,[3,4] essential for splicing progression. A first small molecule probe, selected from known compounds[5] revealed to bind group II introns at the catalytic core (co-crystallographic experiments). To unequivocally confirm the binding mode of this hit compound, a brominated analogue probe was synthesized and revealed detailed position within the target through X-ray anomalous scattering,[6] thus clarifying some essential features of this Intron-targeting small molecule class.

Our data exemplify the power of RNA binders to mechanistically probe vital cellular pathways, and prove that splicing machines can specifically recognize small molecules. This work puts solid bases for the rational design of novel compounds selective for the evolutionarily-conserved RNA cores, including bacterial and organellar introns, and also the human spliceosome, validated target for congenital diseases and cancer. Starting from this pioneering work, a medicinal chemistry campaign towards these ends has been undertaken in our laboratories.

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FC5

DISCOVERY OF PYRAZOLE-PYRIMIDINES AS NOVEL CDK7 INIHIBITORS: SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION

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The latest findings in anticancer research have showcased the potential to identify new therapeutic targets through the analysis of aberrations in crucial functional genes. Due to its involvement in the dysregulation of transcription and cell cycle progression, cyclin-dependent kinase 7 (CDK7) has emerged as a highly pursued avenue for anticancer therapeutics. Remarkably, in patients with highly aggressive and metastatic lung cancers, the inhibition of CDK7 results in a notable downregulation of gene transcription and induces cell cycle arrest.[1-3] In our efforts to design novel CDK7 inhibitors, we conducted an evaluation of the structures of various CDK7 inhibitors developed thus far. Interestingly, we observed that the pyrazolo[1,5-*a*]pyrimidine scaffold is present in several inhibitors such as BS-181. On the other hand, the indole moiety has also been reported in covalent CDK7 inhibitors such as THZ1, SY-1365 and in SY-5609 ATP-competitive inhibitor. Therefore, we have successfully combined these two fragments into a single structure of type **1**, where two identical indole units are linked to the central ring of pyrazolo[1,5-*a*]pyrimidin-7-amine . We then conducted a biological evaluation of a novel library of synthesized derivatives, focusing on the assessment of their potential to inhibit CDK7 and to explore their anticancer properties in lung cancer models.

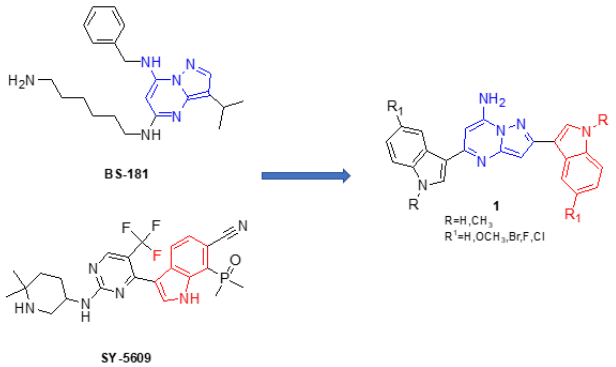

Figure 1.

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FC6

IN VITRO CHEMICAL AND METABOLIC STABILITY AND IN VIVO PHARMACOKINETIC STUDIES OF A CONFORMATIONALLY RESTRICTED RETIGABINE ANALOGUES AS NOVEL NEURONAL KV7 CHANNEL ACTIVATORS

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Kv7 K+ channels represent attractive pharmacological targets for the treatment of different neurological disorders, including epilepsy. Retigabine (RET) is the prototype Kv7 activator, but several drawbacks are likely responsible for its limited clinical efficacy which can be summarized as follows: (*i*) poor selectivity for Kv7 subtypes; (*ii*) short half-life. RET is metabolized by phase-II enzymes, with little involvement of the cytochrome P450 system. Consequently, RET has fast clearance kinetics and requires dosing three times a day; (*iii*) poor brain penetration. Because of its limited lipophilicity, the brain−plasma concentration ratio of RET is rather low, thus requiring posological schemes at relatively high doses; and (*iiii*) chemical instability. One of the main clinical concerns over RET is its tendency to cause retinal and mucocutaneous blue-grey discoloration; although the mechanism for this toxic effect remains poorly understood; one hypothesis is that light exposure may cause photodegradation and oxidation of RET's aniline ring, which may lead to dimer formation, partly in conjunction with melanin, which would be responsible for the abnormal pigmentation light-exposed tissues[1].

To possibly overcome some of these limitations, we have designed and synthesized a small library of novel conformationally restricted RET derivatives and characterized some of these compounds with respect to potency, selectivity. An integrated mass spectrometry-based strategy to evaluate photostability, in Vitro Metabolism and in vivo brain/plasma distribution allowed us to identify a novel compound was identified (compound **60**)[2]. When compared to RET, compound **60** showed higher potency and efficacy as a Kv7 channel activator in vitro, no photoinduced dimer formation, higher brain/plasma ratio, and longer plasma half-life in vivo. All these pharmacokinetic and pharmacodynamic properties likely contributed to its increased antiseizure activity in vivo with respect to the parent compound. Overall, our results suggest that compound **60** might represent a promising lead compound for further development of novel Kv7 activators for clinical use in epilepsy and other hyperexcitability diseases.

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FC7

NOVEL PYRROLE TUBULIN ASSEMBLY INHIBITOR AS ANTICANCER AGENT INDUCING FERROPTOSIS

Puxeddu, M.;[a] La Regina, G.;[a] Sebastiani, J.; D'Ambrosio, M.;[a] Nalli, M.;[a] Liu, T.;[b] Hamel, E.[c] and Silvestri, R.[a]

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Apoptosis-induced programmed cell death has always been recognized as the only pathway to take off cancer cells. Recently, ferroptosis has been investigated as non-apoptotic programmed cell death mechanisms with potential to overcome the block of apoptosis in mutant cancer cells. Ferroptosis programmed necrosis is mainly triggered by peroxidation of extra-mitochondrial lipid arising from accumulation of iron-dependent ROS, which are produced by excessive iron deriving from abnormalities of major redox systems and aberrant iron metabolism.[1] It has been established that ferroptosis is an effective approach in anticancer therapy for the eradication of residual or resistant cancer cells. A lot of evidence supports the perspective applications of ferroptosis in GBM therapies. On the other hand, OC cells showed susceptibility to ferroptosis since an excess iron gets overloaded in tumor-initiating cells after overexpression of transferrin receptor 1 and decrease of the level of iron efflux pump ferroportin.[2]

In this work, we replaced the 1-(methylphenyl) group of 1 with a pyridine or pyrimidine ring and kept fixed hydrogen, phenyl or furan-2-yl (the latter heterocyclic ring has shown a tight interaction with the colchicine binding site at position 4 of the pyrrole). We discovered that a new aroyl diheterocyclyl pyrrole (ARDHEP) of this series, compound **15** (scheme 1), whose mechanism is the inhibition of tubulin polymerization, exhibited the hallmarks of ferroptosis (figure 1) rather than conventional apoptosis expressed by **1**, meanwhile the angiogenic effect remained similar for both compounds.[3]

Scheme 1

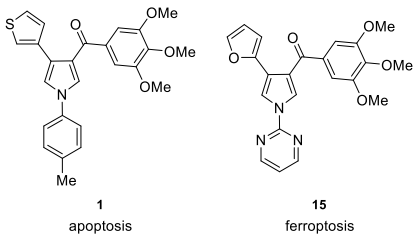
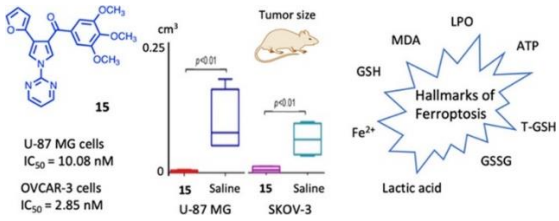


Figure 1



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FC8

THE RS6077 AS NEW INDUCER OF CELL DEATH IN A LYMPHOMA TUMOR *IN VIVO*

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In previous works, the introduction of an amino group at position 3' on the 1-phenyl ring of the tubulin polymerization inhibitor 1 provided a derivate (2) which showed a strong inhibition of the MDR cell lines and of the Hedgehog signaling pathway (Figure 1). The replacement of a carbon with a nitrogen atom seemed to affect potency, selectivity, and physicochemical properties, including improved water solubility. Thus, we designed and synthesized the new pyrrolic congener RS6077 as a potential inhibitor of tubulin polymerization. The compound inhibited tubulin polymerization with a submicromolar IC_{50} value (0.28 μM), and it was a strong inhibitor of the binding of [^{3}H]colchicine to tubulin. The novel agent showed both *in vitro* and *in vivo* anti-tumor activity against lymphoma models.[1] Indeed, all cell lines tested from aggressive lymphomas were found sensible to this inhibitor in the nanomolar range, with IC_{50} values between 25 and 45 nM. The RS6077 also exhibited activity against a xenograft ABC-DLBC model without observed toxicity in the treated animals. Importantly, the the derivate arrested cells in the G2/M phase of the cell cycle in both transformed and non-transformed cell lines. Importantly, the time-lapse video-recordings revealed that the new derivate in non-neoplastic cells triggered a shorter mitotic arrest without activating the death-in-mitosis pathway, however in HeLa transformed and in lymphoid-derived transformed AHH1 cell lines cells the death was effectively induced during mitotic arrest in cells that fail to complete mitosis. which could represent a significant therapeutic advantage of compound. Together these findings suggest that derivative RS6077 has potential as novel therapeutic agent to treat lymphomas.

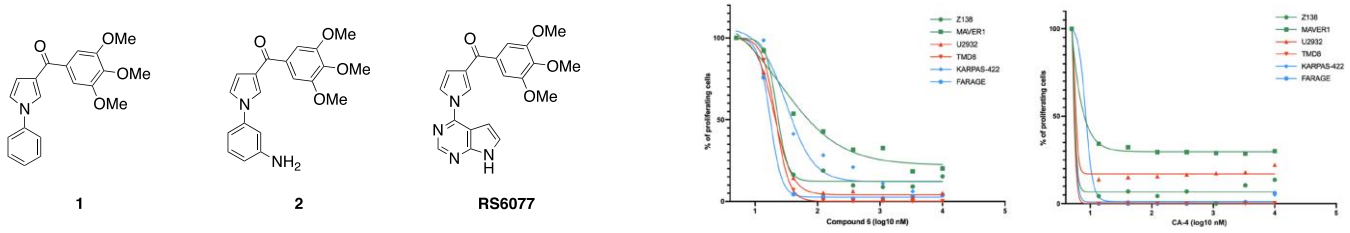

Figure 1. New tubulin polymerization inhibitors. Inhibition of proliferation by RS7077 (6) and CA-4 in lymphoma cells.

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FC9

MOLECULAR HYBRIDS BETWEEN ANTIGLAUCOMA DRUGS AND H_2S DONORS: SYNTHESIS AND IN VITRO H_2S RELEASING PROPERTIES

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Glaucoma is a worldwide leading cause of irreversible vision loss. It represents a group of neurodegenerative diseases characterized by the progressive death of retinal ganglion cells (RGC). Elevated intraocular pressure (IOP) is the main clinical manifestation of glaucoma.[1] A role for hydrogen sulfide (H_2S) has been found in the physio-pathological processes of the eye. It has been shown that, over the capacity in decreasing IOP facilitating aqueous humor outflow, H_2S scavenges reactive oxygen species, upregulates the cellular antioxidant glutathione and protects RGCs from toxicity.[2]

Based on this evidence, the aim of this project was to synthesize new molecular hybrids that combine H_2S-releasing molecules with antiglaucoma drugs to obtain a new class of prodrugs displaying synergic/complementary effect. The molecular hybrids were synthetized by coupling antiglaucoma drugs, namely brinzolamide, betaxolol and brimonidine, with H_2S donors. The H_2S-releasing properties of the new compounds were evaluated by amperometric approach. Moreover, the intracellular release of sulfide was assessed in Human Primary Corneal Epithelial Cells by spectrofluorometric measurements. The general procedure for the synthesis of the molecular hybrids was based on the condensation of the selected drugs with H_2S releasing moieties by mean of an acetic or a succinic spacer. Brinzolamide, betaxolol and brimonidine were 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). The amperometric assay demonstrated that in the absence of L-Cys all the compounds showed a completely negligible release of H_2S. On the other hand, the preincubation with an excess of L-Cys (4 mM) improved the gasotransmitter release from brinzolamide and brimonidine molecular hybrids. The fluorometric assay indicated that all brinzolamide and brimonidine derivatives generated a significant H_2S release, while the betaxolol hybrids evoked only a mild increase in the intracellular sulfide levels.

In conclusion, novel molecular hybrids between brinzolamide, betaxolol and brimonidine with H_2S releasing moieties were synthetized. The new molecular entities were tested for their sulfide releasing properties via amperometric and fluorometric assays. Experimental data showed that compounds **1c** (brinzolamide-HBTA), **1d** (brinzolamide-HPI) and **3d** (brimonidine-HPI) determined the most favorable H_2S releasing properties, consisting in an intense and long-lasting production of H_2S both in the aqueous solution (in the presence of L-Cys) and in the intracellular environment.

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FC10

NOVEL AZEPINO[4,3-*b*]INDOLE DERIVATIVES AS LIGANDS OF THE CANNABINOID-ACTIVATED ORPHAN RECEPTORS GPR18 AND GPR55 WITH POTENTIAL AGAINST NEURODEGENERATIVE DISEASES

Samarelli, F.,[a] Purgatorio, R.,[a] Catto, M.,[a] Carrieri, A.,[a] Nicolotti, O,[a] Mahardhika, A., Vielmuth, C.,[b] Müller, C.E.,[b] Altomare, C.D.,[a] and de Candia, M.[a]

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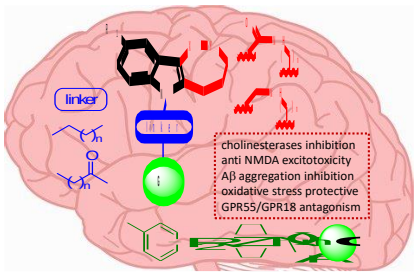
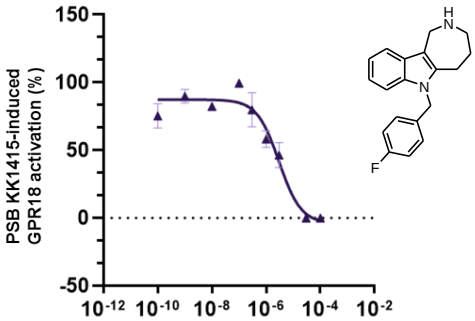
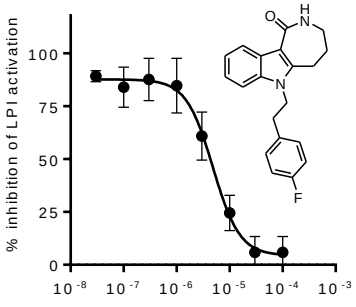
Despite the progresses in understanding the multifactorial nature of Alzheimer's disease (AD), the efforts to develop effective drugs failed.[1] Multi-target directed ligands (MTDLs), i.e., molecules able to simultaneously address more than one target involved in the disease onset and progression,[2] may be endowed with potential as AD modifying agents. We recently reported tetrahydroazepino[4,3-*b*]indole (THAI) as a novel scaffold of selective butyrylcholinesterase (BChE) inhibitors, showing in vitro additional protective activity against NMDA-induced excitotoxicity,[3] and PET images obtained after i.v. administration of a ^{18}F-labeled THAI active derivative, allowed us to assess in mice its distribution into the brain and peripheral organs.[4]
Herein, we report as fragments linked to the THAI nucleus can tune the whole neuroprotective profile, thus returning compounds that also behaved as β-amyloid protein aggregation breakers, and even more interestingly as antagonists of orphan cannabinoid-activated G protein-coupled receptors GPR18 and GPR55, which are involved in neuroinflammation associated to AD.[5] Chemical and biological results, along with SARs and molecular modeling results, will be presented and discussed.







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FC11

GREEN AND AFFORDABLE KETOAMIDES SYNTHESIS AS VERY POTENT BROAD-SPECTRUM COVs MAIN PROTEASE INHIBITORS: X-RAY STRUCTURE AND BIOLOGICAL PROFILE

Alberico, A.;[1] Pelliccia, S.;[1] Storici, P.;[2] Costanzi, E.;[2] Tramontano, E.;[3] Corona, A.;[3] Cerchia, C.;[1] Summa, V.;[1]

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After almost two years from its first evidence, the COVID-19 pandemic, caused by the SARS-CoV-2, continues to afflict people worldwide, highlighting the need for multiple antiviral strategies. While vaccinations provide invaluable protection, the approved drugs Remdesivir and Molnupinavir targeting the CoV RdRp and Nirmatrelvir targeting the CoV Main Protease (M^{pro} or $3CL^{pro}$) suffer of modest efficacy or suboptimal PK properties. The M^{pro} is a cysteine protease that has been identified as a solid target for antivirals, due to its importance in viral maturation, high conservation among different CoVs and lack of a human homolog. [1] To develop novel M^{pro} inhibitors, multicomponent reactions were used, and the Passerini reaction-amine deprotection-acyl migration (PADAM) oxidation route was employed to synthesize small peptidomimetics compounds with a ketoamide warhead that act as covalent reversible inhibitors. [2,3] Biochemical assays revealed that these peptidomimetics had an IC_{50} in nanomolar/low micromolar range on SARS-CoV-2 and MERS M^{pro}, and antiviral cell-based assays demonstrated low micromolar EC_{50} and no detectable cytotoxicity. X-ray co-crystal structures of protease–inhibitor complexes were also determined, revealing the molecular determinants of the interaction and providing key hints for further development for small molecules inhibitors.

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FC12

N-ADAMANTYL-ANTHRANIL AMIDE DERIVATIVES: NEW SELECTIVE LIGANDS FOR THE CANNABINOID RECEPTOR SUBTYPE 2 (CB2R)

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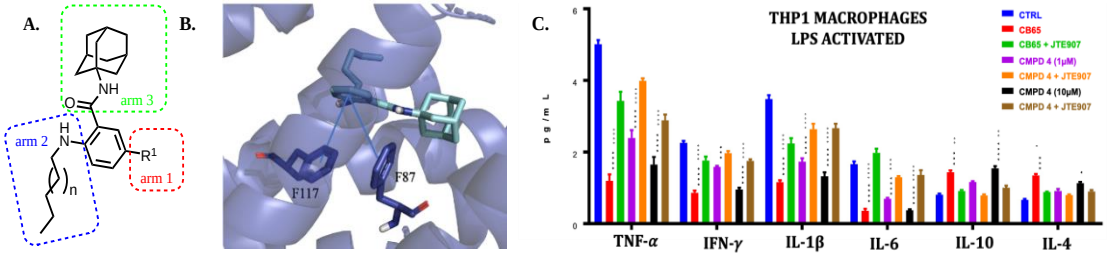
Cannabinoid 2 receptor (CB_2R) is a G-protein coupled and together with Cannabinoid 1 receptor (CB_1R) forms the Endocannabinoid system (ECS). CB_2R is expressed especially in cells of the immune system and play a key role in disorders based on an inflammatory state, such as neurodegenerative diseases, neuropathic pain and cancer. For this reason, the anti-inflammatory and immunomodulatory potentials of CB_2R ligands are emerging as a novel therapeutic approach. Starting from latest released CB_2R crystal structures, we designed, synthesized, and evaluated a series of new *N*-adamantyl-anthranil amide derivatives as CB_2R selective ligands (Figure 1A).[1] Interestingly, these compounds displayed a high affinity for human CB_2R along with an excellent selectivity respect to CB_1R. The best compound in terms of CB_2R affinity were also evaluated for their functional profile and molecular docking simulations provided a sound rationale by highlighting the relevance of the arm 1 substitution to prompt CB_2R action (Figure 1B). Moreover, the modulation of the pro- and anti-inflammatory cytokines production was also investigated to exert the ability of the best compounds to modulate the inflammatory cascade (Figure 1C).

Figure 1. General structure of our *N*-adamantyl-anthranil amide derivatives (A), top-scored docking poses returned by docking simulations (B) and modulation of the best compound on cytokine production (C).



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FC13

APPLICATION OF CLICK CHEMISTRY IN THE DEVELOPMENT OF NOVEL ANTILEISHMANIAL PROTEOLYSIS TARGETING CHIMERAS

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Click chemistry is a flourishing approach in the drug discovery and development. The Nobel Prize in Chemistry awarded in 2022, is just the confirmation of this trend. The term "click chemistry" refers to fast, simple, wide in scope reactions giving high yields after an easy product isolation.[1] It is not surprising that this strategy is successfully used in the synthesis of many molecules, such as Proteolysis-Targeting Chimeras (PROTACs). PROTACs are heterobifunctional compounds comprising three elements: a ligand for a protein of interest (POI), an E3 ligase (E3) recruiting element, and a linker. PROTACs may show potential for treating infectious diseases.[2,3] In our research, we are focused on **the development of first-in-class antileishmanial PROTACs**. Leishmaniasis is a vector-borne disease affecting around 1 million patients annually, mainly in tropical and subtropical areas. The lack of an effective therapy calls for the urgent search of novel drugs.[4] As a POI, we chose trypanothione reductase (TR) enzyme that is a validated antileishmanial target.[5] Taking into account that the relevant length and rigidity of the linker is a crucial for the formation of the ternary POI-PROTAC-E3 ligase complex, and consequently, the degradation by the proteasome system,[6] we decided to synthesize triazole-based PROTACs, by using click chemistry (**Figure 1**). Herein, we present the design, synthesis and biological evaluation of such new series of PROTACs as potential antileishmanial agents.

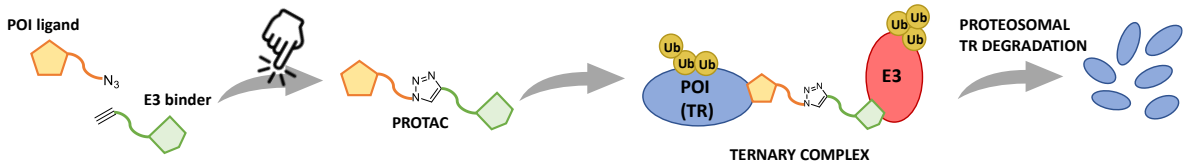

Figure 1. Click chemistry concept in the development of antileishmanial PROTACs**.** This work is supported by FISR2019_03796 "PROLEISH (PROTACs to treat leishmaniasis)".

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FC14

STRUCTURAL FUNCTIONALIZATION OF TRI-SUBSTITUTED PYRAZOLE DERIVATIVES AND PRELIMINARY EVALUATION OF THEIR ANTIMALARIAL ACTIVITY

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The increasing prevalence of drug-resistant *Plasmodium falciparum* strains led to an urgent priority in the development of new antimalarial drugs.[1] In this scenario, several pyrazole derivatives have been reported as antimalarial agents in the last years.[2,3] Recently, novel Tri- and Tetrasubstituted pyrazole derivatives (compounds **1**, Figure 1) showed interesting antimalarial properties being endowed with micromolar IC_{50} values against Chloroquine (CQ)-sensitive D10 and CQ-resistant W2 *Plasmodium* strains.[4] To further extend the structure activity relationships around these promising compounds, we designed and synthesized a new series of 3-phenyl-4-cyano pyrazoles bearing different basic chains at position 5 (compounds **2**, Figure 1). Briefly, the novel derivatives were prepared by sequentially reacting benzoylacetonitrile with carbon disulfide and iodomethane in the presence of sodium hydride. The so obtained thioketal **A** was substituted with the proper amine, leading to the formation of intermediates **B**, that were cyclized with hydrazine to afford the desired pyrazoles.

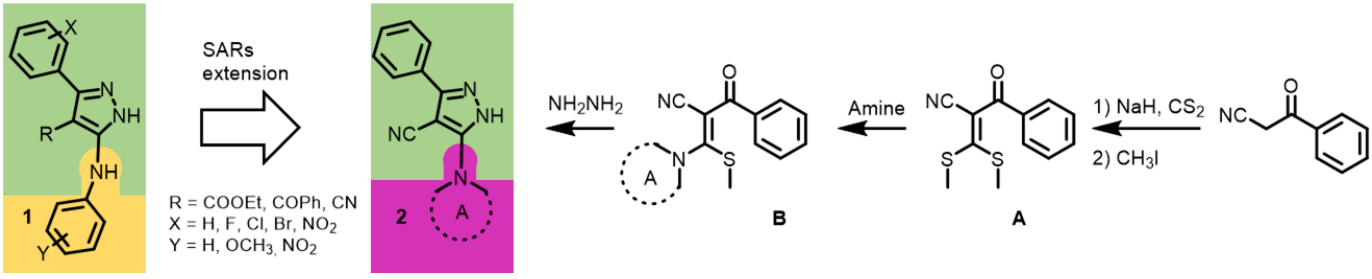

Figure 1. SARs extension of previous reported compounds **1** and synthesis of pyrazoles **2**.

All the newly isolated compounds were tested against D10 and W2 *Plasmodium* strains and their cytotoxicity was evaluated on normal fibroblast cells. Interestingly, the novel pyrazoles **2** showed micromolar IC_{50} values especially against CQ resistant W2 strain, resulting more effective than their analogues **1.** Additionally, in preliminary tests the novel derivatives did not show any significant cytotoxicity on fibroblast cell line.

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FC15

NATURE-INSPIRED ANTIBACTERIAL AGENTS: SYNTHESIS AND BIOLOGICAL ACTIVITY OF EUGENOL DERIVATIVES AGAINST *H. pylori* STRAINS

Melfi, F.;[a] Ammazzalorso, A.;[a] Tanini, D.;[b] Mencarelli, N.;[a] D'Agostino, I.;[a] Marinacci. B;[a] Sisto, F.;[c] and Carradori, S.[a]

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Essential oils are widely recognized as an interesting source of bioactive compounds. Eugenol is an important component of clove essential oil, with broad-spectrum antibacterial activity.[1,2] The occurrence of antibiotic resistance is the most important factor responsible for the failure of *H. pylori* eradication therapy. In this context, eugenol showed a remarkable antibacterial profile when tested *in vitro* against *H. pylori*.[3] In this work, we investigated different chemical modifications on eugenol scaffold, generating three different series of derivatives: in series *A*, a diazoaryl function was added at the *ortho* position; in *B*, the phenolic group was alkylated or incorporated into a carbamate or ester moiety; in *C*, the allylic portion was replaced by a differently substituted tail, including an epoxide ring, alcohol or chalcogen-bearing chains [**Figure 1**]. The antibacterial susceptibility of *H. pylori* strains was evaluated against the reference NCTC 11637 strain and three drug-resistant clinical isolates. Interestingly, all the derivatives showed lower minimal inhibitory concentration (MIC) values against *H. pylori* NCTC 11637 than the parent compound and maintained their antibacterial activity also against the resistant strains, exerting a bactericidal effect. *In vivo* toxicity in a model of *G. mellonella* was also performed for the most active derivatives.

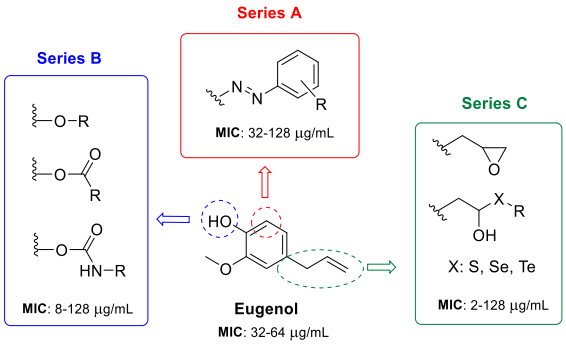

Figure 1. Eugenol structure and chemical modifications in this study.

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FC 16

EBSELEN ANALOGUES AS HUMAN NEUTHOPHIL ELASTASE (HNE) INHIBITORS AND ANTIOXIDANT AGENTS

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Human neutrophil elastase (HNE) is a serine protease stored in neutrophils and is involved in several respiratory diseases, such as chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), acute lung injury (ALI) and cystic fibrosis, but also in cancer, rheumatoid arthritis and psoriasis[1,2]. Moreover, the involvement of the enzyme in Covid-19 has been recently highlighted, since the excessive HNE proteolytic activity in the lungs is responsible for the exacerbation of the severe respiratory complications related to the disease[3,4]. Our research group has been working in the field of HNE inhibitors for many years, obtaining potent compounds with nanomolar activity. These compounds are characterized by the presence of an N-CO function which attacks the OH group of Ser195 in the catalytic triad. In further project development, a series of benzo[*d*][1,2]selenazol-3(2H)-ones as Ebselen analogues have been synthesized. The aim was to obtain compounds with anti-HNE and antioxidant effects, the latter due to selenium, leading to compounds with dual action, which are particularly attractive for the treatment of respiratory diseases (Figure 1).

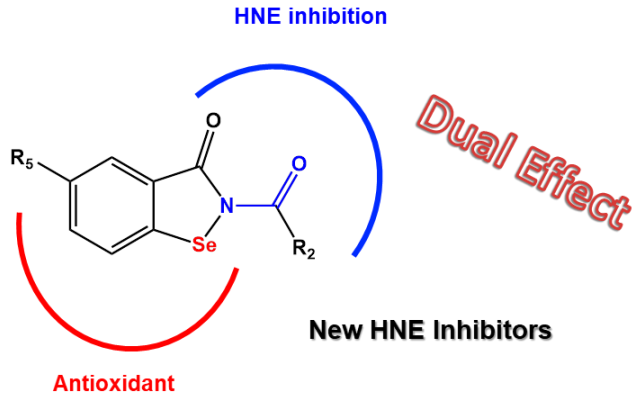

Figure 1. Aim of the work

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FC17

DUAL ANTA-INHIBITORS TARGETING PROTEIN KINASE CK1Δ AND A_{2A} ADENOSINE RECEPTOR USEFUL IN NEURODEGENERATIVE DISORDERS

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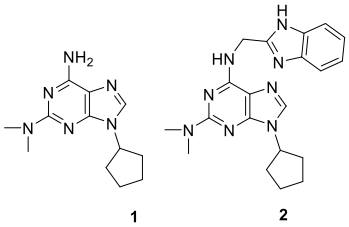
The A_{2A} adenosine receptor (A_{2A}AR) has been recently implicated in the neuroinflammation observed in neurodegenerative diseases.[1] In the event of a decreased functionality or brain injury, A_{2A}AR expression is amplified in microglial cells, leading to abnormal signal transduction, including enhanced release of proinflammatory cytokines, resulting in excessive production of ROS and oxidative stress.[2] In this context, inhibitors that target A_{2A}AR and casein kinase 1 delta (CK1δ) hold promise as a novel approach to treat neurodegenerative disorders and have the potential to exhibit a combinational or even synergistic therapeutic effect.

Figure 1. *Novel A_{2A}/CK1δ dual anta-inhibitors.*



The present report aims to present the results of the evaluation of the activity of two novel A_{2A}/CK1δ dual anta-inhibitors *in vitro* and to assess their intestinal absorption in *ex vivo* (Figure 1)[3]. Experiments on N13 microglial cells showed that these compounds effectively counteracted inflammation induced by a cytokine cocktail (CK), as a consequence of their A_{2A}AR antagonism. In this experiment compound **2** exhibiting higher activity than compound **1** at concentrations of 7.5 and 4.5 µM, respectively, compensating for CK damage. Separate studies have proven that these ligands prevent the CK damage acting through CK1δ inhibition, resulting in an improved cell viability. Compound **2** also displayed a strong antioxidant effect: the amount of nitrite formed after treatment was even lower than in the control without CK. The everted gut sac model and LC-MS were used to measure the compounds in mucosal and serosal media, providing a comprehensive understanding of their transport. HPLC analysis showed that both compounds can cross the barrier (up to 47% drug absorbed for **1** and 23% for **2**), making them promising candidates for gastrointestinal delivery.

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FC18

FIRST-IN CLASS SPECIFIC INHIBITORS OF THE MITOCHONDRIAL DEACYLASE SIRTUIN 4

Fabbrizi, E.[1]; Pannek, M.[2]; Al Halabi, Z.[3]; Tomaselli, D.[1]; Menna, M.[1]; Fiorentino, F.[1]; Robaa, D.[3]; Weyand, M.[2]; Puhlmann, M.[2]; Tomassi, S.[4]; Barreca, F.[5]; Tafani, M.[5]; Zaganjor, E.[6]; Haigis, M.[6]; Sippl, W.[3]; Steegborn, C.[2*]; Rotili, D.[1*]; Mai A.[1*].

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Sirtuin 4 (Sirt4) is a NAD^+-dependent protein lysine deacylase located in mitochondria known to regulate fatty acid metabolism, insulin release, and apoptosis by modulating different enzymes, such as glutamate dehydrogenase (GDH) and pyruvate dehydrogenase (PDH).[1,2] Sirt4 is a potential drug target for treating cancer and metabolic diseases, but no potent and selective inhibitors have been reported so far. Hence, we set out to identify Sirt4-specific small molecule inhibitors. Testing top candidates from a target-based virtual screening led to compounds **2** and **15**, with IC_{50} values of 46 and 67 µM, respectively. Starting from these two compounds we developed a series of derivatives leading to compounds **60** and **69** with IC_{50} values of 0.19 and 16 µM, respectively (Figure 1). Kinetic analyses suggest that the compounds to compete with the acyl peptide substrate but not with NAD^+, consistently with docking models. They further show preference for Sirt4 over other isoforms, with **69** being selective over Sirt1-3 and Sirt5 at 50 µM. Interestingly, **60** and **69** dose-dependently increased the activities of GDH and PDH, two enzymes inhibited by Sirt4, in C2C12 cells without affecting cell viability. Moreover, **69** suppressed adipocyte differentiation, known to be modulated by Sirt4.[3] In summary, our study identified first-in-class potent and specific Sirt4 inhibitors as chemical tools useful for studying the Sirt4 biology and its therapeutic potential in various pathological conditions, including metabolic diseases and cancer.

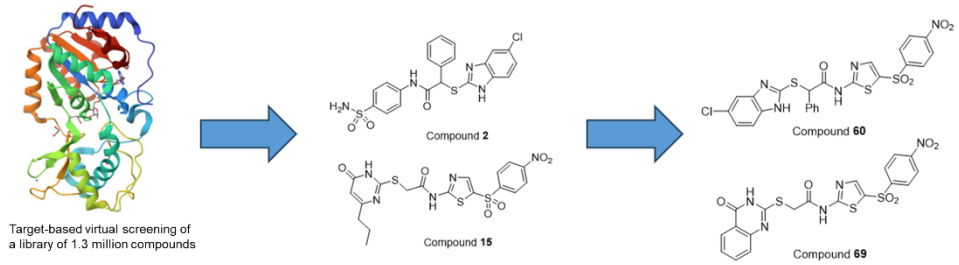

Figure 1. Development of Sirt4 inhibitors.

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POSTER
presentations

POSTER 1

LONG NON-CODING RNA AND COMPUTATIONAL TECHNIQUES: CHALLENGING COMBINATION FOR THE DISCOVERY OF NEW COMPOUNDS WITH ANTICANCER ACTIVITY

Rocca, R.;[a,b] Artese, A.;[a,b] Amato, J.;[c] Richter, S.N.;[d] Tassone, P.[a] and Alcaro, S.[a,b]

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Growing evidence describes the important role of long non-coding RNAs (lncRNAs) in regulating epigenetic mechanisms and gene expression levels, and their dysregulation is closely associated with different diseases such as cancer.[1] In this context, the application of computational tools describing the structures of nucleic acids and their interactions with small molecules is gaining more and more interest. Here, we reported our recent efforts in discovering new small molecules that interfere with lncRNAs through different computational approaches. In particular, we focused our attention on TERRA[2] and MALAT1,[3] leading to the discovery of two new compounds with antitumor activity (Figure 1).

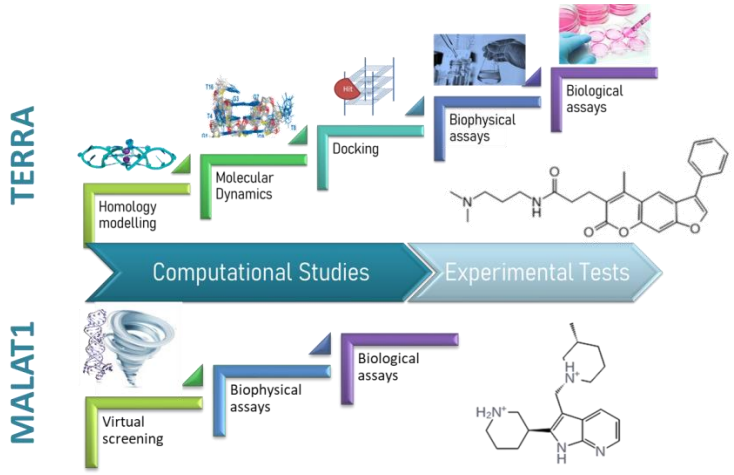

Figure 1. Workflows applied for the discovery of two new small molecules interfering with the lncRNAs MALAT1 and TERRA.

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POSTER 2

DESIGN, SYNTHESIS, MOLECULAR MODELING AND PHARMACOLOGICAL EVALUATION OF NOVEL DIAZABICYCLO[4.3.0]NONANE AND 2,7-DIAZASPIRO[3.5]NONANE AS SIGMA RECEPTORS LIGANDS

Cosentino, G.,[a] Dichiara, M.;[a] Ambrosio, F. A.;[b] Barbaraci, C.;[a] González-Cano, R.;[c] Costa, G.;[d,e] Giordano, G.;[a] Pasquinucci, L.;[a] Marrazzo, A.;[a] Cobos, E. J.;[c] Alcaro, S.;[d,e] and Amata, E.[a]

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As part of our research program aiming at the discovery of novel sigma receptors (SRs), here we present the design and synthesis of 2,7-diazaspiro[3.5]nonane (series 1) and diazabicyclo[4.3.0]nonane (series 2) compounds. The synthesized ligands were evaluated in S1R and S2R radioligand binding assays, and modeling studies were carried out to deeply understand the pose in the binding pocket. Compounds **4b**, **5b** and **8f** had the best binding affinity and thus have been screened for their pharmacological and functional profile by both *in vivo* and *in vitro* assays.

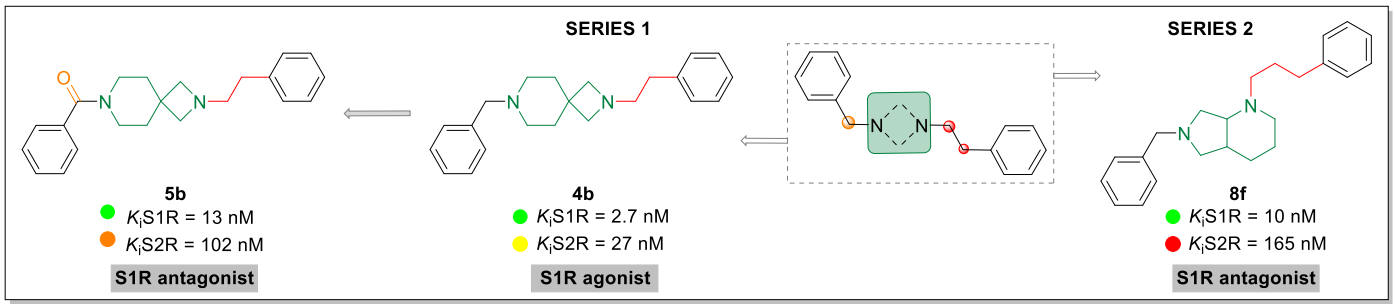

Figure 1. Chemical structures of novel SR ligands with 2,7-diazaspiro[3.5]nonane (**4a**, **4b**) and diazabicyclo[4.3.0]nonane (**8f**) cores.

Compounds **5b** and **8f** showed an antiallodynic effect which was reversed by the selective S1R agonist PRE-084, indicating a clear dependence on the S1R antagonism. Noteworthy, compound **4b** was completely devoid of antiallodynic effect, although it shares the 2,7-diazaspiro[3.5]nonane core as **5b**. Our study might establish the importance of 2,7-diazaspiro[3.5]nonane and of diazabicyclo[4.3.0]nonane cores for the development of novel S1R ligands with specific agonist or antagonist profile.[1]

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POSTER 3

LIGAND-BASED DRUG REPURPOSING STRATEGY AND MULTIPLE BIOPHYSICAL TECHNIQUES IDENTIFIED VIRAL RNA G-QUADRUPLEX BINDERS

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So far, almost all new antiviral therapeutic strategies have focused on targeting viral proteins.[1,2] However, the threat posed by SARS-CoV-2 infection has required exploring also plausible alternative approaches, such as targeting viral RNA and, in particular, its secondary structures.[3,4] Indeed, the folding of specific regions of the viral genomic RNA into certain secondary structures may hinder the viral genome expression and replication by acting as roadblocks for RNA transcription and/or as hallmarks for the attachment of RNA processing machinery. Among these structures are the G-quadruplexes, noncanonical four-stranded structures that can be formed by the folding on itself of single-stranded guanine-rich nucleic acid sequences.[5] Critical roles for G-quadruplexes have been described in several viruses, including single-stranded RNA viruses.[6,7] For example, the single-stranded RNA genome of SARS-CoV-2 contains some G-quadruplex-forming G-rich elements which are promising drug targets.[4] In this work, we performed a ligand-based pharmacophore virtual screening of FDA approved drugs to find candidates targeting such RNA structures.[8] Further *in vitro* assays identified three drugs as emerging RNA G-quadruplex binders. Our results lay the basis for further studies aiming to evaluate the antiviral activity of such drugs, while the methodological approach employed will certainly impact medicinal chemistry approaches for targeting of viral RNA, even beyond SARS-CoV-2.

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POSTER 4

SYNTHESIS AND IN VITRO PHARMACOLOGICAL EVALUATION OF 2-HYDROXYPROPYL-4-ARYLPIPERAZINE DERIVATIVES AS SEROTONINERGIC LIGANDS WITH ANTIPROLIFERATIVE ACTIVITY

Andreozzi, G.;[a] Ambrosio, M.R.;[b] Magli, E.;[a] Corvino, A.;[a] Maneli, G.;[c] D'Ambrosio, F.;[c] Bassolino, R.;[c] Caliendo, G;[a] Formisano, P.;[b,c] and Fiorino F.[a]

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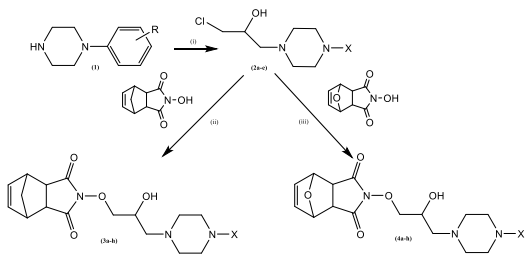
Pharmacological regulation of the 5-HT system has a great therapeutic potential, and therefore it is the subject of intense studies.[1] One of the most interesting 5-HT receptors, involved in mediating 5-HT biological effects, is the 5-HT_{1A} receptor that is involved in a wide range of psychiatric disorders, and even in the proliferation of human tumor cells (PC3). Preclinical studies have shown that prostate carcinoma cell lines PC3 and breast carcinoma cell lines MCF-7 express serotonin receptors[2] and the treatment with specific antagonists inhibits cell growth in dose-dependent way[3]. In this study we report the synthesis of two series of compounds, embodying norbornene and exo-N-hydroxy-7-oxabicyclo [2.2.1] hept-5-ene-2,3-dicarboximide nuclei, 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, hydroxyalkyl chain, and 4-substituted piperazine) known to be critical for affinity to 5-HT_{1A} receptors and the proper selection of substituent led to compounds with high specificity and affinity toward serotoninergic receptors. Moreover, the compounds displaying better affinity and selectivity binding profiles towards 5-HT_{1A} and 5-HT_{2A} receptors were selected to be tested by in vitro and in vivo assays to determine their functional activity. Hence, PC3 cells have been incubated in the presence of raising concentrations (range 5 – 50 μM) of the selected compounds (3a, 3c,3e, 3g, 4a, 4j) and cell viability has been assessed. IC_{50} for all compounds was identified between 20 and 50 μM. The concentration corresponding to IC50 for each compound was used in further experiments in combination with raising concentrations of docetaxel, a first line drug for androgen-independent prostate cancer (range 2.5 – 20 nM). Interestingly, the combination of compounds 4a and 4j, which were also the molecules with the lowest IC_{50} (20 μM), with docetaxel significantly decreased PC3 cell viability at a higher extent, compared to the effect of both docetaxel and the single compound alone, suggesting a potentiation effect.



Scheme 1.

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POSTER 5

ONION (ALLIUM CEPA L.) LEAVES WASTE VALORIZATION FOR EXTRACTION OF ANTIOXIDANT COMPOUNDS: MICROWAVE-ASSISTED EXTRACTION AND BOX–BEHNKEN DESIGN APPROACH OPTIMIZATION

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By-products of the agri-food sector are still an undervalued important resource, since they still contain large percentage of high value functional substances such as antioxidants, pectin, polyphenols, fibers, among others. The recovery of the above active ingredients with high nutritional and cosmetic value, with the aim of creating a product to be placed on the market as a nutraceutical, cosmeceutical, or functional food, is an integral part of environmental sustainability with a view to intersection and complete circularity between agriculture, food industry and health-products' industry.[1] The production of onion (*Allium cepa* L.), one of the most widely grown vegetables in the world, generates large amounts of waste containing a large number of bioactive compounds. Onion solid waste (OSW) generally includes onion skins, outer fleshy scales, roots, and the apical and basal trimming of bulbs.[2,3] In this study, we developed and optimized an alternative method based on microwave-assisted extraction (MAE) for the recovery and isolation of bioactive compounds from green stalk (leaves) of "Cipollotto Nocerino DOP" onion. A response surface methodology through a Box–Behnken design was applied, and model fit, regression equations, analysis of variance and 3D response curve were developed. Temperature (60–80–100 °C), time (5–15–25 min), extraction volume (6–9–12 mL) and ethanol concentration (40–60–80 % *v/v*) were studied as the major parameters affecting the extraction efficiency and the antioxidant activities. A Box–Behnken design was adopted including 29 experiments with five center points. Under the MAE optimized conditions, the results showed that TPC and FRAP were significantly influenced by temperature, extraction time and extraction volume. Thus, TPC and FRAP varied from 0.76 to 1.43 mg GAE g-1 DW; 8.25 to 14.80 mmol Fe(II)E g-1 DW, respectively. The optimal experimental condition (60 °C; 22 min; 11 mL; 50 % *v/v* EtOH) showed a recovery of TPC and FRAP of 1.35 (mg GAE g-1 DW) and 14.02 (mmol Fe(II)E g-1 DW), respectively. Our results showed that leaves of "Cipollotto Nocerino DOP" are an important source of antioxidant polyphenols.

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POSTER 6

HDAC6 INHIBITORS IN CYSTIC FIBROSIS: THE FIRST PROOF-OF-CONCEPT

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Compelling new evidence highlighted the role of histone deacetylase 6 (HDAC6) enzyme in cystic fibrosis pathogenesis, with particular account of the onset and maintenance of dysregulated inflammatory and fibrotic phenotypes[1]. We have provided the first in vivo proof-of-concept for the efficacy of a selective HDAC6 inhibitor in contrasting inflammatory processes in a CF-related murine model of acute and chronic *P. Aeruginosa (PA)* infection. Three tool compounds were selected for their pharmacokinetic features, selectivity and potency on HDAC6, and for their synthetic feasibility. After their re-synthesis, efficacy in enzymatic and cell assays, and solubility were evaluated in house, confirming literature data, and identifying compound **1**[2] (Figure 1) as the best inhibitor to be tested for its efficacy in the in vivo model. Assessment of **1** on a chronic model of airway infection demonstrated a robust dose-dependent reduction of the recruitment of macrophages in the bronchoalveolar lavage fluid (BALF). Furthermore, Bio-plex Multiplex highlighted a robust reduction of interleukins involved in inflammation (Figure 1). In conclusion, these results confirmed the efficacy of selective HDAC6 inhibition as therapeutic tool in contrasting CF inflammatory processes, encouraging the development of new and more potent and selective HDAC6 inhibitors.

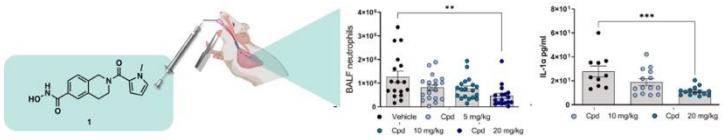

Figure 2. Evaluation of inflammatory cells and markers after seven days of treatment with compound 1 in a chronic PA infection mouse model.

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POSTER 7

FPR2 AGONISTS WITH A TWIST: A SERIES OF UREIDOPROPANAMIDE DERIVATIVES WITH H_2S RELEASING PROPERTIES FOR THE TREATMENT OF NEUROINFLAMMATION

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Alzheimer's Disease (AD) and Parkinson's Disease (PD) are incurable neurodegenerative diseases whose pathology involves several concurrent phenomena, namely protein deposition (Aβ in AD, α-synuclein in PD), neurotransmitter dysregulation, and neuroinflammation. These factors create a situation of chronic oxidative stress that drives neurodegeneration. In recent years, neuroinflammation has especially garnered increasing research interest: it is a complex phenomenon that involves a variety of cell types, receptors, and mediators, and resolving it could unlock disease-altering therapeutic strategies[1].

The research presented herein aims to explore the medicinal chemistry of two important, if somewhat underestimated, players involved in neuroinflammation, namely formyl-peptide receptor 2 (FPR2) and hydrogen sulfide, H_2S. The former is a promiscuous G-protein coupled receptor expressed in macrophages and the microglia, where, depending on the ligand, it can elicit pro-inflammatory or anti-inflammatory signals[2]; the latter is a gas which, although toxic in high doses, has a recognized role as a neuromodulator (regulating calcium influx) and as a neuroprotective agent at low doses[3].

In particular, we have synthesized three series of compounds, by incorporating potentially H_2S-releasing moieties into the pharmacophore of known FPR2 agonists of the ureidopropanamide class[4] (namely by substituting their urea with a thiourea or their amide with a thioamide) or by linking the same structural scaffold with known H_2S releaser ADT-OH. With a few exceptions, these substitutions did not excessively impact FPR2 activity, while they also endowed our compounds with interesting H_2S-releasing properties, thus providing an interesting starting point for future research.

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POSTER 8

SIMPLIFIED 1,2,4-TRIAZINE COMPOUNDS: UNLOCKING ENHANCED ANTITUMOR EFFICACY *VIA* PDK INHIBITION

Carbone, D.;[a] Biancucci, C.,[a] De Franco, M.;[b] Pecoraro, C.;[a] Cascioferro S.,[a] Cirrincione, G.,[a] Parrino, B.,[a] Gandin, V.;[b] and Diana, P.[a]

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Among the various hallmarks of cancer, the deregulation of cellular metabolism has been identified as an essential mechanism that promotes cancer resistance and progression.[1] In the last decade, several research efforts have focused on developing novel agents targeting protein kinases or glucose-metabolizing pathways, highlighting the key role of pyruvate dehydrogenase kinases (PDKs) in cancer metabolisms and suggesting that targeting PDKs would present an exciting therapeutic possibility for cancer treatment. Overexpression of PDKs was indeed observed in different types of metabolically altered cancers, including the highly aggressive pancreatic ductal adenocarcinoma (PDAC), and is frequently related to cancer resistance, invasion, and metastasis.[2] Since PDK inhibitors, known to date, do not possess structural similarities to each other,[3] we thought to use a molecular hybridization approach for developing new compounds with improved efficacy and safety, by combining indole and 1,2,4 triazine pharmacophores in a single structure. The first library of 1,2,4 triazine compounds is characterized by an excellent degree of PDK inhibition, with a subtype selectivity for PDK1 and PDK4 isoforms, and a prominent in vitro and in vivo anticancer activity.[4,5] Given these promising results, we have shifted our attention towards the structural simplification of the triazine-based compounds to avoid the "molecular obesity" of leads. In this study, we present the synthesis, the PDK inhibitory activity and the cytotoxicity in 2D and 3D pancreatic cancer models of a new library of triazine analogs, characterized by a 3-amino-1,2,4-triazine ring substituted with an indole moiety at position 4 and a pyridine at position 5.

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POSTER 9

DESIGN, SYNTHESIS, BIOLOGICAL EVALUATION, AND X-RAY STRUCTURE DETERMINATION OF BROAD-SPECTRUM CORONAVIRUS 3C-LIKE PROTEASE PEPTIDOMIMETIC INHIBITORS

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Since the outbreak of the COVID-19 pandemic, the scientific community is working to find a way to prevent and treat the disease. The approval of vaccines, monoclonal antibodies, and restrictions during the pandemic have limited the spread of SARS-CoV-2. Nevertheless, repurposing campaigns and focused medicinal chemistry programs led to the approval of only three direct-acting antivirals (DAAs). Specifically, remdesivir (iv) and molnupiravir (oral administration) are repurposed nucleotide inhibitors of the viral RNA-dependent RNA polymerase with moderate efficacy.[1] The identification of nirmatrelvir, a reversible peptidomimetic covalent inhibitor, is the most significant step forward since it is the first approved inhibitor of SARS-CoV-2 3-Chimotrypsin Like Cysteine protease ($3CL^{pro}$).[2] However, due to its susceptibility to metabolic degradation, in Paxlovid® nirmatrelvir is co-dosed in combination with ritonavir, a cytochrome inhibitor, to allow an acceptable oral dosing regimen. Considering the limited therapeutic options, the incapacity to provide vaccinations in developing countries, and the emergence of several viral variants, the discovery of DAAs highly active against SARS-CoV-2 and CoVs family is still necessary to enrich the current clinical arsenal against COVID-19. $3CL^{pro}$ is a cysteine protease having Leu-Gln as preferred and unique P2–P1 sequence.[3] It is highly conserved in the active site across CoVs and variants, has a pivotal role in the viral life cycle, and is absent in human cells. This profile makes $3CL^{pro}$ a very attractive target for the identification of SARS-CoV-2 DAAs and Pan-Coronavirus inhibitors.[3] We report a series of tripeptidomimetics as covalent reversible inhibitors behaving an aldehydic warhead acting as cysteine trap, a Gln mimetic at P1, and modified P2-P3 residues.[4] Particularly, functionalized proline residues were inserted at P2 to stabilize the β-turn like bioactive conformation in order to modulate the affinity. We describe rational design, synthesis, biological evaluation in biochemical and phenotypic assays, and X-ray crystallography of representative compounds in complex with the protein target. The most promising inhibitors displayed low/sub-nM potency against the $3CL^{pro}$ of SARS-CoV-2 and MERS-CoV and inhibited viral replication of three human CoVs (SARS-CoV-2, MERS-CoV, and HCoV 229) in different cell lines, showing a broad-spectrum activity against CoVs.

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POSTER 10

IN SILICO ASSISTED DESIGN OF PYRROLE-BASED DERIVATIVES AS CB2 AGONISTS: A COMPREHENSIVE ANALYSIS OF CB2R ROLE IN MEMORY AND COGNITION

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Cannabinoid receptors, CB1 and CB2, are highly expressed in the central nervous system (CNS), where they mediate the biochemical activities of endogenous cannabinoids.[1] Their role ranges from physiological processes such as appetite regulation,[2] neuroprotection,[3] and energy metabolism[4] to pathological processes such as cancer,[5] pain, and neuroinflammation.[6] Furthermore, CB2 receptor is upregulated under neuroinflammatory, oxidative and ischemic conditions and several agonists are reported to exert neuroprotective activity in models of neurodegenerative conditions.[7] In the present work, we tested a series of molecules from an in-house library, upon filtration by virtual screening protocol, as CB2 agonists. The compounds were initially tested by chemical-physical assays for their ability to bind both CB1 and CB2 receptors. In this way a hit compound was identified, with outmost selectivity over CB2 isoform. This compound was then subjected to a hit-to-lead rational development, designing a new library of analogues. The new compounds were screened for their ability to activate CB2 receptors using functional assays. Compound **70** was selected as the most potent derivative, also showing suitable pharmacokinetic properties, and was then further characterized for its in vivo impact on short-term memory, using a scopolamine-induced amnesia protocol. Moreover, compound **70** was challenged for its capability in modulating neuromodulators levels, using MALDI-imaging technique. Collectively, compound **70** displayed high in vivo effectiveness, suggesting pyrrole scaffold as starting point for further development of new small molecules for the treatment of neuroinflammatory diseases.

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15th Young Medicinal Chemists' Symposium Nuove Prospettive in Chimica Farmaceutica
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POSTER 11

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PROTEOLYSIS TARGETING CHIMERAs (PROTACs™) OF G9a LYSINE METHYLTRANSFERASE

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Over the past years, Targeted Protein Degradation (TPD) has emerged as an attractive strategy to modulate the activity of target proteins by inducing their selective degradation. PROteolysis TArgeting Chimeras (PROTACs™) are heterobifunctional molecules able to hijack the target protein to ubiquitin-proteasome machinery (UPS) for their selective degradation. From a structural point of view, a PROTAC™ compound comprises a ligand for the protein of interest (POI) joined by a linker to a ligand capable of binding an E3 ubiquitin ligase.[1] On the strength of the good results obtained in the development of PROTACs™ for epigenetic targets, we aimed to extend this approach to the lysine methyltransferase G9a.[2] This protein is an epigenetic writer capable of mono- and dimethylating lysine residues of histones substrates, mainly H3K9, but also non-histone proteins. Through its activity, this protein is involved in several cellular processes such as cell differentiation, embryonic development, cognitive and adaptive behavior. In addition, high expression levels of G9a have been found in many types of human cancers, including breast, ovarian carcinoma, head, and neck squamous cell carcinoma.[2,3]
Herein, we report the design, synthesis, and biological evaluation of PROTAC™ degraders of G9a protein by employing the cereblon/cullin 4A and VHL/cullin 2 degradation systems and the quinazoline-based **UNC0638** as G9a ligand (Figure 1).

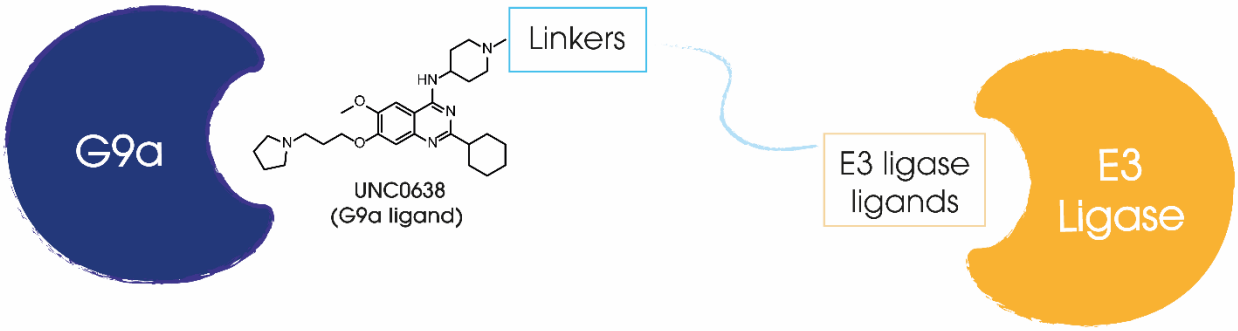

Figure 1. Design of G9a PROTACs™

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POSTER 12

SIGMA/HDACi DUAL-LIGANDS: A NEW POTENTIAL APPROACH FOR ANTICANCER THERAPY

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Designing and discovering compounds as dual-target inhibitors is challenging to synthesize new, safer, and more efficient drugs than single-target drugs, especially to treat multifactorial diseases such as cancer. The simultaneous regulation of multiple selective targets might represent an alternative approach to optimize patient compliance and tolerance, minimizing the risk of target-based drug resistance. To this end, we conceived for the first time the design and synthesis of dual-ligands σR/HDACi to evaluate possible employment as innovative candidates to address this complex disease.[1] Among all synthesized compounds screened for several tumoral cell lines, compound **6** resulted the most promising candidate as an antiproliferative agent with an IC_{50} of 0.9 µM on the HCT116 cell line (**Figure 1**) and no significant toxicity to normal cells. Studies of molecular docking, which confirmed the affinity over σ_1R and a pan-HDACs inhibitory behavior, support a possible balanced affinity and activity between both targets.

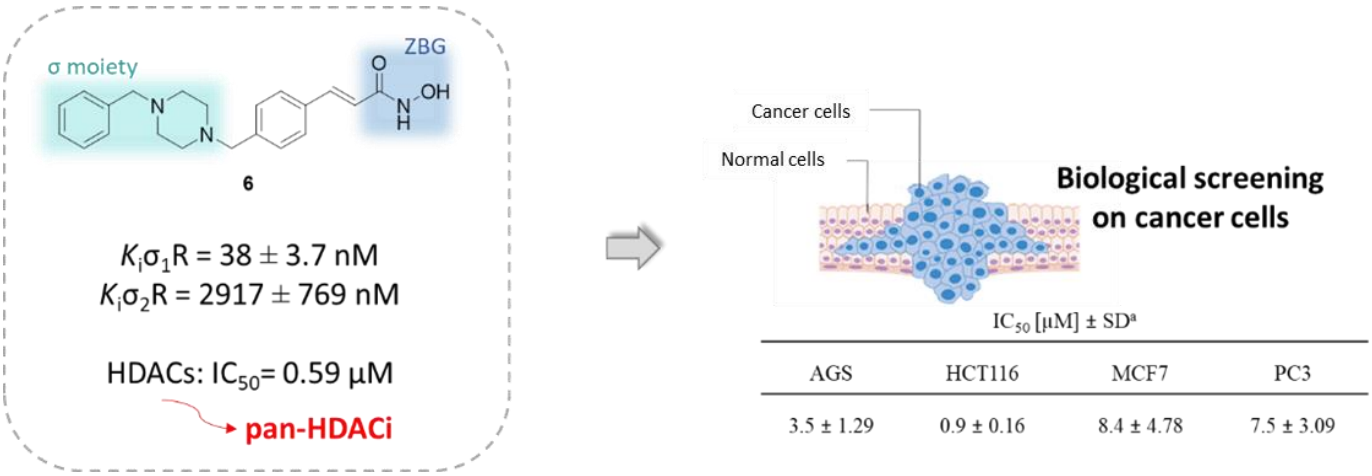

Figure 1. Biological activity for compound 6**.**

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POSTER 13

DEVELOPING H2S DONATING HYBRIDS AS NOVEL OPPORTUNITY IN MULTIFACTORIAL DISEASES

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Since the discovery of hydrogen sulfide (H2S) as the third gasotransmitter, among to nitric oxide (NO) and carbon monoxide (CO)1, the relevance of this endogenous mediator as a signaling molecule in biological systems has been unanimously confirm2. In this context, several lines of research suggest the use of exogenous H2S (as H2S donors) for the treatment of a variety of multifactorial diseases.

As most diseases are multifactorial in nature, the hybridization strategy is being pursued with increasing intensity, which has resulted in improved outcomes in disease models, especially for disorders such as neurodegenerative, neoplastic, metabolic and inflammatory diseases, where multi-target strategies are a promising alternative to the classical "one target-one drug" design approach.

Based on this strategy, we developed novel multitarget molecules, combining well-known H2S-donor moieties with pharmacologically active compounds, such as anti-inflammatory agents, including glucocorticoids3,4 and non-steroidal anti-inflammatory drugs (NSAIDs)5, antihyperglycemic drugs, including sitagliptin, and anti-Parkinson drugs, including pramipexole. The obtained hybrids were tested in different disease models and resulted to be able to enhance the desired pharmacological effect or reducing the incidence of adverse effects of the original drug.

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POSTER 14

NOVEL *N*-NORMETAZOCINE DERIVATIVES WITH SIGMA-1 RECEPTOR ANTAGONIST PROFILE AS POTENTIAL ANALGESICS FOR INFLAMMATORY PAIN MANAGEMENT

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To date, treating chronic inflammatory pain represents one of the unmet clinical needs for patients, as the conventional therapies cause several side effects. Recently, new targets involved in inflammatory pain modulation have been identified; among these is the sigma-1 receptor (σ1R). It acts as a molecular chaperone able to modulate the function of different proteins. The σ1R modulation of analgesia was proposed by Chien and Pasternak, who identified σ1R as a potent anti-opioid endogenous system[1]. Several hypotheses explain the involvement of σ1R in inflammatory pain modulation[2]. Furthermore, selective σ1R antagonists have demonstrated analgesic efficacy in acute and chronic inflammatory pain models[3]. So, based on these evidences, a series of novel *N*-normetazocine derivatives have been designed and synthesized to investigate the pivotal role of *N*-normetazocine stereochemistry in their pharmacological fingerprint.

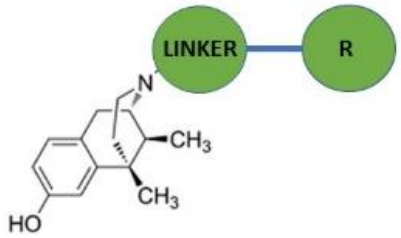

Figure 1 General structure of novel synthesized compounds

Intermediates and final compounds have been properly purified through flash chromatography. The structural characterization was determined by ^{1}H-NMR, ^{13}C-NMR. In vitro, their affinity profile *versus* sigma receptors was performed through competition binding assays. Some assayed compounds showed a relevant σ1R affinity, and the most promising molecules will be tested in vivo by the Formalin test. Furthermore, molecular modeling studies are in progress to analyze the binding mode and the interactions between the new ligands and sigma receptors.

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POSTER 15

SYNTHESIS AND CHARACTERIZATION OF NEW A_{2A} ADENOSINE RECEPTOR PARTIAL AGONISTS

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Adenosine (Ado) is an endogenous purinergic nucleoside widely distributed in all organisms where participates in a large number of physiological and pathophysiological processes through the interaction with four receptor subtypes: A_1, A_{2A}, A_{2B} and A_3, called adenosine receptors (ARs). In particular, A_{2A}ARs are involved in the microglia-astrocytes cross-talk and their activation seems to stimulate exosome production/release, mechanism that facilitates the communication among glial cells.[1] Adenosine signaling is crucial also in the microglia-astrocyte communication during brain inflammatory state that occurs over Glioblastoma development. The activation of the A_{2A}AR present in a specific T cells class (Teff), mediated by Ado, leads to an increase of cAMP and an inhibition of cytokines production, which triggers to a cascade of events culminating in endo vesicle (EV) production.[2] These EV, inhibiting the immune system, have a prominent pro tumoral role, therefore the release inhibition could represent a promising therapeutic strategy. A partial agonist can display both agonistic and antagonistic effects since it is able to enhance deficient systems while simultaneously blocking excessive activity, like in tumor environment.[3] Starting from the consideration that the introduction of a 1-hexynyl and a phenylethylthio chains in position 2 of Ado leads to derivatives that possesses high affinity for the A_{2A} receptor subtype,[4] and that the 2-hexynylAdo and its 8 alkyl amino substituted derivatives have been reported as partial A_{2A} agonists,[5] in this work, new 2,8-disubstituted Ado derivatives were designed, synthetized and biological characterized. To obtain the desired compounds, commercial guanosine was converted through 5 synthetic steps in the versatile intermediate 8-bromo-2-iodo-Ado, in which it was possible to selectively introduce different alkylamine chains at the 8 position and then different substituents at the 2 position.

The compounds were tested in displacement assays of specific radioligands on recombinant human ARs, expressed on Chinese hamster ovary cells (CHO), with the aim of evaluating their affinity and activity on the A_1, A_{2A}, A_{2B} and A_3 ARs. Results confirmed that they behave as partial agonists of the A_{2A}AR, with intrinsic activity values below 0.5. Studies of these molecules in models of glioblastoma are in progress in order to validate their utility in this type of cancer.

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POSTER 16

LOW MOLECULAR WEIGHT CATIONIC LIPOPEPTIDES AS INNOVATIVE STRATEGY FOR FIGHTINGH ANTIMICROBIAL RESISTANCE

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Antibiotic Resistance is a global clinical issue caused by the widespread and incorrect use of antibiotics1. In this perspective, antimicrobial peptides (AMPs) are an emerging class of chemotypes that could represent new agents against resistant bacterial infections with a broad spectrum of activity. Unfortunately, AMPs have several limitations, including poor stability to serum proteases and cytotoxicity to host cells, as well as high production costs2. To address these issues, several chemical modifications have been investigated. Among them, the reduction of the AMPs size3 and the conjugation of lipid moieties are emerging as promising strategies to improve antimicrobial activity and metabolic stability, minimizing the host cells toxicity3. Herein, a library of short lipopeptides was *de novo* designed and synthesized. These are endowed with a common peptide core consisting of Arg-Pro-Arg, the Arg interacts with the negatively charged bacterial counterpart, while the Pro, a well-known structuring amino acid, reduces the freedom degree of a spacer or a cationic amino acid bearing the fatty acid. The so obtained lipopeptidomimetics were preliminary tested *in vitro* and a structure-guided design was employed to improve their activity and reduce their toxicity. Among all the synthesized tested lipo-peptidomimetics we identified some promising results, which displayed MIC values in the low-µM range against gram (-) and gram (+) bacteria and low-to-moderate toxicity versus host cells.

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POSTER 17

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL POTENT MUSCARINIC RECEPTOR ANTAGONISTS BEARING THE 1,4-DIOXANE NUCLEUS

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The muscarinic acetylcholine receptor (mAChR) family consists of five closely related members (M_1-M_5) with different molecular and signaling properties.[1] mAChRs mediate several functions in the central nervous system[2], as well as in the periphery.[3] The 1,4-dioxane ring has recently demonstrated to be a bioversatile scaffold for the development of compounds binding mAChRs. In particular, we have demonstrated that it is possible to obtain mAChR agonists or antagonists, depending on the nature of the substituent in position 6 of the 1,4-dioxane nucleus. Specifically, the presence of bulky substituents in position 6 allowed us to obtain potent antagonists, including the 6,6-diphenyl derivative **1**. In addition to quaternary ammonium compounds, such as **1**, ligands bearing a tertiary amine (**2**) (Figure) also proved to act as antagonists at mAChRs, probably by binding in the cationic form.[4]

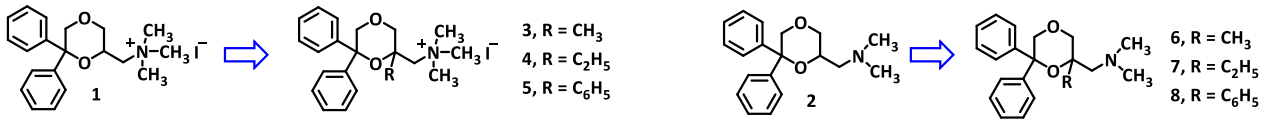

Figure. Chemical structures of compounds **1**-**8**.

In this study, we have designed and synthesized the quaternary ammonium compounds **3**-**5** and the tertiary amines **6**-**8** (Figure) by inserting in position 2 of the potent 6,6-diphenyl-1,4-dioxane mAChR antagonists **1** and **2**, respectively, substituents of different steric bulk, with the aim of evaluating their role on the mAChR affinity. Moreover, considering the critical role of chirality in the mAChR affinity of 1,4-dioxanes, the enantiomers (+)-**6** and (-)-**6** were also prepared and studied. The most interesting result was obtained with the 2-methyl derivative **6** and its enantiomers which, surprisingly, in binding studies exhibited a biphasic curve, showing two distinct pK_i values for all the five mAChRs. Molecular modelling studies were performed to rationalize this unexpected experimental observation.

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POSTER 18

NOVEL BIOCOMPATIBLE COPPER COMPLEXES POTENTIALLY USEFUL AS ANTIVIRAL AGENTS

Del Bello, F.;[a] Giorgioni, G.;[a] Piergentili, A.;[a] Quaglia, W.;[a] Caviglia, M.;[b] Del Gobbo, J.;[b] Pellei, M.;[b] Santini, Carlo;[b] Morelli, M.B.[a]; Zeppa L.[a]

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Copper complexes have gained great interest over the years for their versatile applications in medicinal inorganic chemistry.[1] We have recently reported Cu(I) and Cu(II) complexes with heteroscorpionate ligands conjugated with different biomolecules, including a noncompetitive NMDA receptor antagonist and the anticancer drug lonidamine,[2] endowed with cytotoxic activity toward a panel of human tumor cell lines. Here we describe the synthesis and biological evaluation of new Cu(I) and Cu(II) complexes functionalized with the antiviral agent amantadine (Figure). The bis(pyrazol-1-yl)acetic acid LH was selected as a bifunctionalizable coordinating agent for the preparation of the ligand L^{CS7} due to the k^3-NNO coordination behavior of bis(azol-1-yl)acetates and to the presence of a carboxylic function suitable for the coupling with the primary amine group of amantadine. For the synthesis of the novel Cu(I) complexes, phosphane co-ligands, such as the hydrophilic PTA and the lipophilic PPh_3, were selected to stabilize copper in +1 oxidation state and to confer different solubility properties to the corresponding complexes.

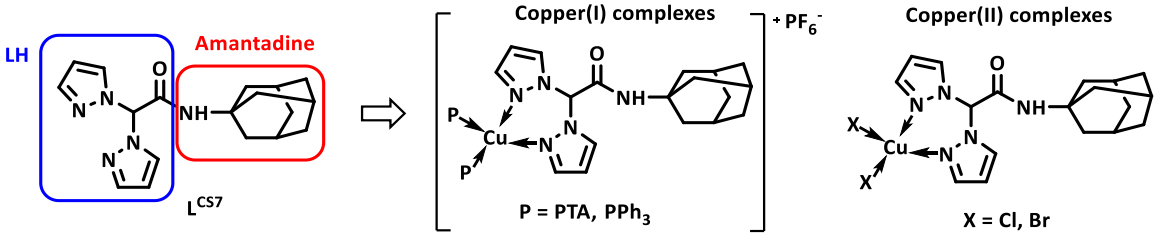

Figure. Chemical structures of ligand L^{CS7} and the relative Cu(I) and Cu(II) complexes.

All the newly synthesized complexes were primarily evaluated by MTT cell viability assay for their cytotoxicity on two mammalian immortalized cell lines, such as HaCaT and Vero E6 cells, and the most biocompatible ones were selected to be tested for their *in vitro* antiviral activity by using lentiviral vectors expressing luciferase in Vero E6 cells.***Acknowledgements*****:** *NextGenerationEU - D.M. 737/2021 "INVIRCuM" University of Camerino FAR 2022 PNR.*

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POSTER 19

ULTRASOUND-ASSISTED PEPTIDE NUCLEIC ACIDS SYNTHESIS (US-PNAS)

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PNA are artificial DNA-like molecules with an uncharged peptidomimetic backbone consisting of nucleobases-functionalized N-(2-aminoethyl) glycine (AEG) units in place of the sugar-phosphodiester moieties[1] (Figure 1). Solid Phase Synthesis (SPS) represents the most efficient approach for synthesizing oligomers with high purity and good yields, nevertheless, issues like the low coupling yields in sequences with a high content of sterically hindered purines, still affects PNA synthesis. Considering the pharmacological potential applications of PNA, we propose an improved and easily accessible protocol for PNA oligomers, by employing ultrasonication in all the steps of the PNA synthesis (US-PNAS) (Figure 2). Theuse of ultrasounds in chemistry has received notable attention, due to their ability to enhance the reactionrates and the product yields, thanks to the cavitation phenomenon. Our research team has recently reportedthe development of an US-assisted Solid Phase Peptide Synthesis strategy[2], that enhanced the synthesis of difficult peptides without increasing the main side reactions. Herein, we investigated the best coupling reagent, the lower stoichiometric excess of reagents, and the lower coupling time comparing the conventional with the US-supported synthesis. We demonstrated that the application of the US-PNAS approach improved the crude product purities and the isolated yields of different PNA, including small or medium-sized oligomers (5-mer and 9-mer) complex purine-rich sequences (like Guanine homoligomers and the telomeric sequence, TEL-13) and longer oligomers (like the 18-mer anti-IVS2-654 PNA and the 23-mer anti-mRNA 155 PNA)[3]. Noteworthy, the ultrasound-assisted strategy is compatible with the commercially available PNA monomers and well-established coupling reagents and only requires the use of an ultrasonic bath, which is a simple equipment generally available in most synthetic laboratories.

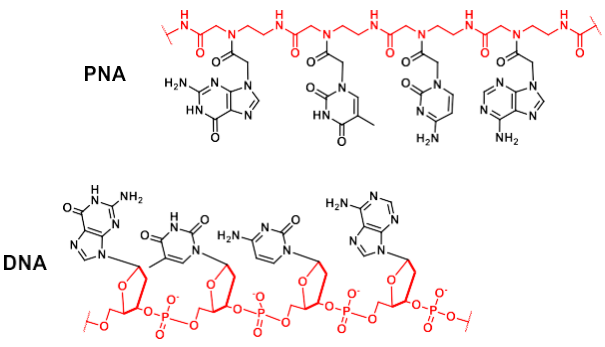

Figure 1. Representative comparison between PNA and DNA

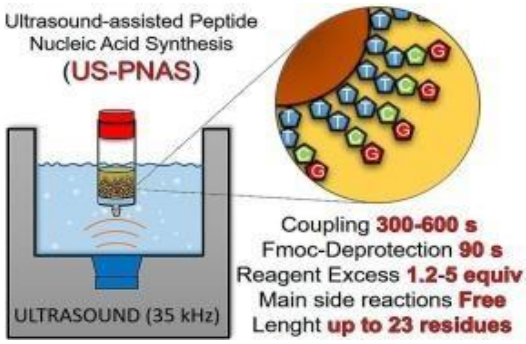

Figure 2. Ultrasound-assisted Peptide Nucleic Acid Synthesis

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POSTER 20

DEVELOPING A COMPUTATIONAL WORKFLOW TO RETRIEVE SELECTIVITY LIGANDS FOR SIGMA 1 AND SIGMA 2 RECEPTORS

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Sigma receptors 1 (S1R) and 2 (S2R) represent an area of active research due to their multifaceted roles in various physiological and pathological processes. Since S1R and S2R are not homologous, they exhibit differences in their pharmacological properties and structure reflecting in the biological functions of the two subtypes. Indeed, S1R is an attractive target for neurological disease treatments, while S2R is a significant target for the development of antitumor agents.[1]

However, despite having different folds, their binding pockets exhibit similarities by positioning functionally equivalent amino acids in appropriate spatial locations. This clarifies how two very different receptors can possess comparable profiles for recognizing ligands that overlap closely, bearing a positive charge central nitrogen flanked by aromatic and/or liphophilic moietis. [2,3]

In this context, a computational workflow was implemented to identify the critical differences required for obtaining ligands with significant selectivity.

GBPM (Grid Based Pharmacophore Model) and LigandScout were applied to search for selective S1R or S2R ligands and to highlight the most relevant interacting residues of both targets.[4,5] Subsequently, taking into account the well-known phisycalchemical properties of SRs ligands, a virtual screening procedure was carried out to retrieve potential hit compounds. Finally, Glide docking simulations were perfomed to investigate the target molecular recognition of the most promising previously selected compounds.[6]

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POSTER 21

RATIONAL DESIGN OF ZONULIN INHIBITOR AT1001 DERIVATIVES AS POTENTIAL ANTI SARS-COV-2

Di Matteo, F.; [a] Di Micco, S.; [b] Rahimova, R.; [c] Sala, M.; [a] Scala, M. C.; [a] Vivenzio, G.; [a] Musella, S.; [a,b] Andrei, G.; [d] Remans, K.; [e] Mammri, L.; [c] Snoeck, R.; [d] Bifulco, G.; [a] Campiglia, P.; [a,b] Marquez, J. A.; [c] Fasano, A. [b,f]

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Anti SARS-CoV-2 vaccines are addressing the global pandemic, nevertheless, the development of new therapeutics remains critical in controlling the viral spreading. [1,2] The current most promising strategies involve the use of monoclonal antibodies, although small molecules remain the most investigated option, considering the cost and complications deriving from monoclonal antibodies administration. [3,4] Here, we describe the development of new tripeptide derivatives of AT1001 active against SARS-CoV-2 M^{pro}. Molecular docking studies drove the design and synthesis of a small series of compounds that were then filtered by FRET enzymatic assays, leading to the identification of compound **4** as the most active one. X-ray crystallography studies demonstrated that compound **4** interaction with M^{pro} involved the formation of a covalent bond. *In vitro* antiviral analysis showed that **4** exhibited an improved activity against SARS-CoV-2 M^{pro} compared to the lead compound AT1001. In addition, its efficacy was evaluated in Vero cells, using different viral variants (Wuhan, UK, South African variants). Although compound **4** showed an inhibitory activity against Wuhan and UK variants, it was unresponsive against the South African one; so, additional structural modifications led to compound **58**, that showed a significant antiviral activity against all SARS-CoV-2 variants and a valuable safety and a good pharmacokinetic profile after *in vivo* administration. The encouraging results obtained led us to consider compound **58** a starting point for the development of new series of compounds as adjuvant drugs for the treatment of SARS-COV-2 infections.

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POSTER 22

BIOISOSTERIC REPLACEMENT OF AN UREIDO FUNCTION BY HETEROCYCLES AS A DESIGN STRATEGY OF NOVEL POTENT PRO-RESOLVING FORMYL PEPTIDE 2 RECEPTOR (FPR2) AGONISTS

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The resolution of inflammation (RoI) is a physiological process that occurs during the inflammatory response to terminate inflammation and restore tissue morphology and function. Numerous pathological conditions, including neurodegenerative diseases, are characterized by chronic and unresolved inflammation as a common feature. The stimulation of the RoI, therefore, could offer a new therapeutic opportunity to treat pathologies with a chronic inflammation status.[1] A critical role during this process is played by the formyl peptide 2 receptor (FPR2), a GPCR modulated, endogenously, by several Specialized Proresolving Mediators, such as lipoxins and resolvins.[2] We identified potent non-peptidic FPR2 agonists with an ureidopropanamide scaffold, such as AMS-21 (**Figure 1**), having anti-inflammatory, pro-resolving, and neuroprotective properties in both in vitro and in vivo models of neuroinflammation. The ureido function in these compounds is crucial for interacting with the receptor. Still, it is related to their low solubility in the aqueous media and their potential toxicity in vivo due to the formation of primary aromatic amines.2 Therefore, we bioisosterically replaced the ureido function with aromatic heterocycles (**Figure 1**). We report here on the rational design, synthesis, computational studies, and preliminary biological evaluation of a new set of compounds. The new derivatives' physicochemical and in vitro pharmacokinetic properties will also be discussed.

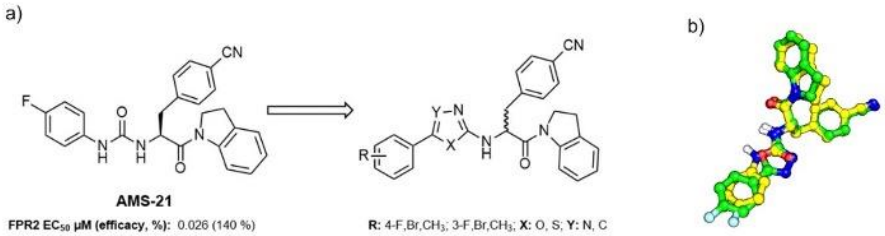

Figure 1: a) Bioisosteric replacement of the ureido function; b) Overlapping of AMS-21 and an oxadiazole derivative.

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POSTER 23

COUPLING OF VIBRATIONAL SPECTROSCOPY AND CHEMOMETRIC ANALYSIS FOR ASSESSMENT OF VIRGIN COCONUT OIL ADULTERATION

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In recent years, virgin coconut oil (VCO) has taken on an important role as a functional food, thanks to the discovery of some biological and healthy properties such as antioxidant, anti-inflammatory, antihyperlipidemic, and antibacterial activity due to some substances such as phenols and tocopherols.[1] VCO is produced by cold pressing, as room temperature prevents degradation processes and ensures low acidity. The commercial price of VCO is about ten times higher than that of conventional vegetable oils, making it a potential target for adulteration. In this study, the use of Fourier Transform Infrared (FTIR) spectroscopy combined with Multivariate Curve Resolution - Alternating Least Squares (MCR-ALS) methodology was evaluated to verify the purity or adulteration of VCO with reference to several low-cost commercial oils such as sunflower (SO), maize (MO) and peanut (PO) oils.[2]

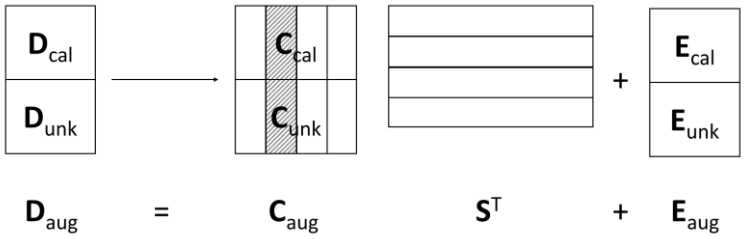

Figure 1. MCR-ALS equation when correlation constraint is applied.

To assess VCO adulteration, control charts were developed using MCR-ALS score values calculated from a data set of pure and adulterated oil samples. The quantification of adulteration was evaluated by building a series of calibration models using MCR-ALS with correlation constraints (Figure 1).[3] Different data pre-treatment strategies were tested to best extract the information contained in the sample fingerprints, and the calibration models were optimized using a genetic algorithm (GA) to select the most significant variables. The models gave satisfactory results in an external validation procedure, with prediction errors of less than 4.6% for samples adulterated with sunflower, maize and peanut oils.

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POSTER 24

SYNTHESIS AND PRELIMINARY IN VITRO ANTIPARASITIC ACTIVITY AGAINST TRYPANOSOMA CRUZI OF NEW IMATINIB-BASED ANALOGS

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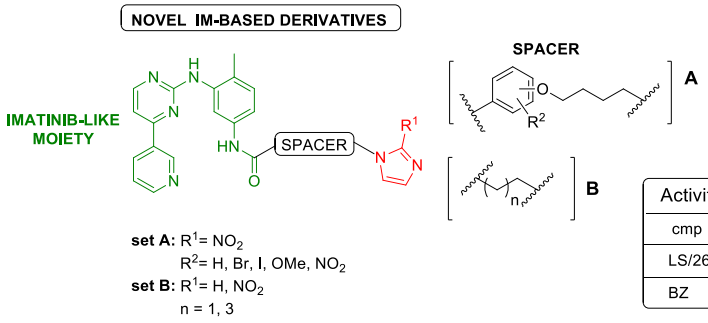
Chagas disease (CD) is a neglected tropical disease (NTD) caused by a parasite named *Trypanosoma* cruzi (*T. cruzi*). CD mainly affects poor regions of Latin America; however, owing to globalization a high interest in NTD drug discovery has emerged in recent years. Currently approved drugs for the treatment of CD, such as benznidazole (BZ) and nifurtimox, are traditional molecules that suffer from significant limitations, including low efficacy during the later chronic phase of the infection, and several side effects; therefore, several efforts have been made to discover new lead compounds offering new therapeutics.[1] Accordingly, we recently performed a phenotypic screening against *T. cruzi* of imatinib (IM) derivatives, originally developed for chronic myeloid leukemia.[2,3] We found that most of these compounds demonstrated superior trypanocidal activity compared to both IM and BZ.[3] With this in mind, here we developed novel IM-based analogs (subset A, Figure 1) bearing the 2-nitroimidazole moiety of BZ, as the replacement of the imidazole one from the previous series. Also, a second set of molecules (subset B, Figure 1) has been obtained by using a different kind of spacer to connect the IM moiety with the imidazole or 2-nitroimidazole nucleus. Newly synthesized compounds were assayed in a *in vitro* phenotypic model of CD to test their anti-*T. cruzi* activity. Preliminary results obtained so far are very encouraging since most of the tested compounds, i.e. LS/26 (figure 1), showed high activity against intracellular forms (Tulahuen strain) of *T. cruzi*, associated with an outstanding selectivity index (SI) over non-infected L929 cells. Further details will be presented at the meeting.



Activity against Tulahuen strain (μM)			
cmp	EC_{50}	EC_{90}	SI
LS/26	0.46	2.43	>435
BZ	1.38	6.18	>145

Figure 1. General structure and antiparasitic activity of the novel IM-based derivatives.

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POSTER 25

NEW DASATINIB AND HEME OXYGENASE 1 INHIBITOR HYBRIDS AS A NOVEL STRATEGY TO TREAT GLIOBLASTOMA

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Glioblastoma (GBM) is one of the most common and aggressive primary brain tumors in adults, occurring with poor prognosis and recurrence. Despite the availability of the approved alkylating agent temozolomide, its efficacy as systemic monotherapy after surgery is still limited; thus, alternative treatments are urgently needed. Recently, a variety of small-molecule inhibitors have been explored as additional therapeutics to improve patient survival, including the dual Src/Abl kinase inhibitor dasatinib (DASA), evaluated in a Phase 2 trial in patients with recurrent GBM (i.e., RTOG 0627).[1] Surprisingly, no satisfactory clinical outcomes have been achieved likely due to an inadequate delivery or accumulation in the tumor. Heme oxygenase 1 (HO-1) is an inducible enzyme isoform, responsible for the catabolism of the heme group which is overexpressed in many cancer cell types. Interestingly, high levels of HO-1 have been detected in human GBM cell lines, while the augmented enzyme activity is likely to be involved in the growth and progression of the tumor.[2] Therefore, to optimize the chemotherapy of DASA efficacy against GBM, here we described the synthesis and preliminary *in vitro* anticancer activity of novel hybrid compounds. Particularly, in this newly proposed multitarget approach we selected our previously discovered potent HO-1 inhibitors[3] as a counterpart to be coupled with DASA. According to the mutual prodrug strategy, DASA-based hybrids (i.e., TC7, TC8, and TC9, Figure 1) were obtained by conjugating DASA with arylethanol imidazoles through a succinic linker. The novel synthesized hybrids, as well as the combination of the corresponding parent compounds, were tested against human glioblastoma cancer cells (U87MG). Of note, all hybrids showed similar or higher activity on cell viability compared to both the parent drugs and the combo, supporting their further investigation as potential anticancer agents for GBM.

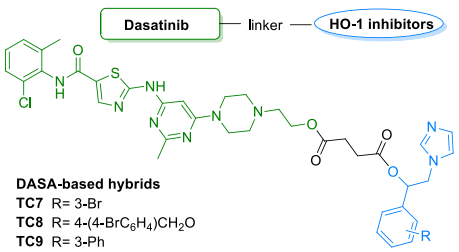

Figure 1. Chemical structure of newly synthesized DASA-based hybrids.

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POSTER 26

STABILITY PROFILE OF ANTICANCER MONOCLONAL ANTIBODY FOR PERSONALIZED TARGET MOLECULAR THERAPY

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In the last decade, significant breakthroughs have been achieved in cancer therapy by applying monoclonal antibodies (mAb) as they are able to directly target cancerous cells. Unfortunately, several drawbacks have yet to be overcome such as drug resistance and poor chemical or physical stability of the antibody glycoprotein. Several parameters and conditions, including the structure of the proteins, temperature and exposure to light, affect mAb stability.[1] The main process related to chemical degradation is the oxidation of some amino acid residues, such as methionine and cysteine.[2] Variations in temperature or pH can induce the unfolding of proteins, leading to a direct loss of mAb functions and favoring their aggregation. Herein, as part of a research project[3] aimed at the development and industrialization of innovative drugs for personalized molecular therapy, the stability profile of trastuzumab was evaluated under different oxidative and light exposure conditions. In accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) rules concerning stability studies applied to pharmaceutical formulations[4], a solution of trastuzumab solution (10 mg/mL) has been exposed to different experimental conditions. The degradation profile has been monitored by UV spectrophotometry by applying multivariate curve resolution (MCR) analysis to the spectral data. Three samples have been prepared by diluting the starting trastuzumab solution with aqueous solutions at pH 3.5, 5 and 8 prepared with the addition of HCl or NaOH, respectively. The analysis was performed at different time intervals up to 2 hours. The results of these preliminary studies demonstrate that the stability of the antibody solution decreases as the pH increases and that with weakly acidic pH, the aggregation phenomena are less present. When the prepared formulations are exposed to light, a decrease of the stability is observed.

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POSTER 27

BIOISOSTERES OF COUMARIN-BASED MTDLs: BIOLOGICAL ACTIVITY PROFILE AND DRUG-LIKENESS STUDY

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The cure for Alzheimer's disease (AD) is a health priority due to the lack of effective therapies[1]. Because of the complex AD pathogenesis, our effort focused on the discovery of multitarget directed ligands (MTDLs). Hopefully, MTDLs act on different biochemical mechanisms to obtain a real disease-modifying effect thanks to synergic or additive activities.In details, our research group has investigated a series of multipotent coumarin-based inhibitors aiming at modulating two key enzymes, namely acetylcholinesterase (AChE) and monoamine oxidase B (MAO B), by following a hybridization strategy[2,3,4]. Structural variations of previously developed hit compounds are currently underway, mainly addressing the drug-like features (i.e., aqueous solubility, membrane permeability). Hit-optimization of compound I (Figure 1) (IC_{50} *h*MAO B = 10 nM; IC_{50} *h*AChE = 120 nM; $Sol_{7.4}$= 13 µM; $logD_{7.4}$= 3.81; CHI=99.3) was based on the introduction of phenyl-ring bioisosteres and on molecular decoration through the introduction of methoxy-group(s) in different positions of the terminal phenyl ring. Moreover, the most promising in vitro multitarget inhibitors were studied in cell models of neuroprotection, after the assessment of inherent cytotoxicity. Finally, inclusion complexes with nanocarriers for efficient CNS drug delivery are also under investigation.

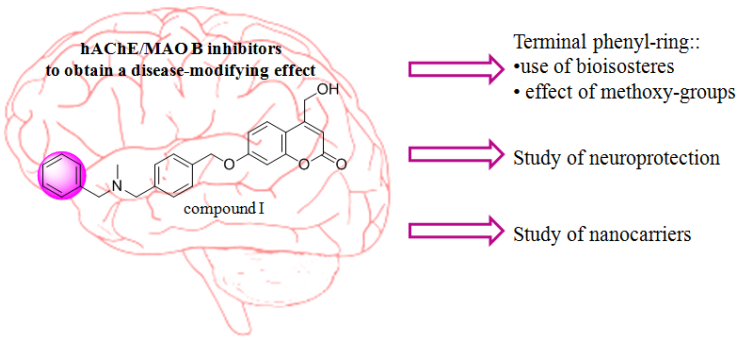

Figure 1. MTDLs coumarin-bearing derivatives workflow.

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POSTER 28

ABLE TO BIND TO CANONICAL AND ALLOSTERIC SITES OF PPARγ

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that control important metabolic functions in the body, mainly implicated in lipid and glucose homeostasis, insulin sensitivity, and energetic metabolism. For this reason, they have been considered suitable targets for the treatment of metabolic disorders comprising obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension.[1] This family includes three receptor subtypes, namely PPARα, PPARγ, and PPARδ, which differ for their tissue distribution, ligand specificity, and also for the downstream effects of their activation. Recent studies have demonstrated that the full activation of these receptors, especially PPARγ, is associated with unwanted effects. To overcome these issues, new research strategies have been undertaken with the aim to obtain new PPAR ligands with reduced side effects and improved beneficial effects. One of these strategies consists in the synthesis of PPARα/γ dual or PPARα/γ/δ pan-agonists, which beneficially alter carbohydrate and lipid metabolism in a coordinated manner. In our previous studies, we identified the novel naphthalenic derivative AL29-26 (Fig. 1), which showed a very attractive PPAR pan-agonist activity profile: potent full agonist on PPARα and partial agonist on PPARγ and δ subtypes. The crystal structures of AL29-26 bound to PPARα and PPARγ showed the crucial role of the gem-dimethyl group of AL29-26 in determining the difference of ligand activity toward PPARα and PPARγ subtypes.[2] This prompted us to focus our attention on this moiety by bringing about a series of chemical modifications, which led to the synthesis of compounds 1−7 (Fig. 1).

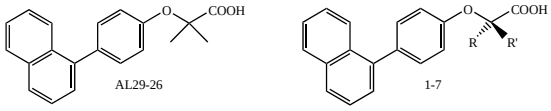

Figure 1.

Given that PPAR activity is strongly affected by the presence of a stereogenic center close to the carboxylic function of α-aryloxy-alkanoic acids, most of these compounds were prepared as enantiomers. Their activity on the three different PPAR subtypes allowed to identify some compounds with an interesting pharmacological profile. In particular, for one of them showing a PPARα/γ dual agonist profile, further biological tests were conducted revealing that this molecule may represent the potential lead of a new class of drugs with better and safer therapeutic effects in the treatment of dyslipidemic type 2 diabetes.

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POSTER 29

hERG STEREOSELECTIVE MODULATION BY MEXILETINE-DERIVED SYMMETRIC AND ASYMMETRIC UREAS: MOLECULAR DOCKING STUDY, SYNTHESIS, AND BIOLOGICAL EVALUATION

Milani, G.;[a] Budriesi, R.;[b] Tavazzani, E.;[c] Cavalluzzi, M. M.;[a] Mattioli, L. B.;[b] Miniero, D. V.;[d] Delre, P.;[e] Belviso, B. D.;[e,f] Denegri, M.;[c] Cuocci, C.;[f] Rotondo, N. P.;[a] De Palma, A.;[d] Gualdani, R.;[g] Caliandro, R.;[f] Mangiatordi, G. F.;[f] Kumawat, A.;[h] Camilloni, C.;[h] Priori, S.;[c,i,l] Lentini, G.[a]

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Long QT syndrome (LQTS) is a disorder of cardiac electrophysiology resulting in life-threatening arrhythmias.[1] The most common subtypes of LQTS are caused by mutations in the human ether-à-go-go-related gene (hERG). The stereoselectivity of a recently discovered mexiletine-derived urea (**1**)[2] was investigated on the hERG potassium channel. According to preliminary in silico predictions, in vitro studies revealed a stereoselective behavior, with the *meso* form showing the greatest hERG opening activity. In addition, functional studies on guinea pig isolated left atria, aorta, and ileum demonstrated that **1** does not present any cardiac or intestinal liability. Due to its overall profile, (*R*,*S*)-**1** (Fig. 1) paves the way for the design and development of a new series of compounds potentially useful in the treatment of both congenital and drug-induced forms of LQTS.

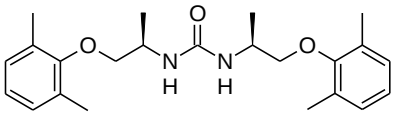

Figure 1. Symmetric mexiletine-derived urea (*R*,*S*)-**1**

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POSTER 30

SYNTHESIS AND IN VITRO EVALUATION OF MELATONIN-H2S DONORS HYBRIDS AS NEUROPROTECTIVE AGENTS

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In recent years, the pineal hormone melatonin has become the focus of considerable interest for its implication in a wide variety of functions involving the circadian, visual, neuroendocrine, reproductive, cerebrovascular, and immune systems[1]. Moreover, melatonin has also been reported to affect blood vessels muscle tone with mainly vasorelaxant effects by concentration-, specific vessel-, and receptor-dependent mechanisms[2]. On the other hand, among potassium channels, voltage-dependent Kv7.4 contributed to the regulation of blood pressure causing vasorelaxation also upon activation of cAMP-mediated signalling[3]. Hydrogen sulfide (H2S) is an ubiquitous gaseous signaling molecule that has an important role in many physiological and pathological processes in mammalian tissues[4]; much evidence has suggested that H2S plays significant beneficial effects for several cardiovascular pathologies including atherosclerosis, ischemia-reperfusion injury, and hypertension[5]; its vasodilatory effects seem to be mediated by modulation of Kv7.4 channels[6]. Considering this evidence, in the present study possible direct effects of melatonin and its newly-synthesized H2S donors hybrids (compounds 1, 2, and 3) on Kv7.4 currents were investigated. The designed Melatoin-H2S donors hybrids have been prepared following the procedure depicted in Scheme 1. The H2S-releasing properties of the new compounds were evaluated by amperometric approach in the presence of L-Cysteine and in a phosphate buffer solution[7]. Amperometric measurement indicated that all compounds release H2S in presence of L-Cys. Patch-clamp technique was also applied using CHO cells transiently expressing Kv7.4 channels 24 h after transfection and exposed to a -80/0 mV ramp protocol. The effects of each compound were measured as % of Kv7.4 current variations versus basal values and the Kv7 activator retigabine (10 µM) was used as a reference compound[8].Results obtained demonstrate that melatonin is a new Kv7.4 activator and that among Melatonin-H2S donors hybrids synthetized, compounds 2 and 3 show stronger potentiating activity on Kv7.4 currents, probably suggesting synergistic effects between melatonin and H2S on Kv7.4 currents.

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POSTER 31

NUSINERSEN INDUCES DISEASE-SEVERITY-SPECIFIC NEUROMETABOLIC EFFECTS IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a neuromuscular degenerative disease caused by homozygous deletions or mutations in the survival motor neuron [1] (SMN1) gene. Although SMA is a prototypical motor neuron disorder, findings in animal models and patients also indicate multiorgan and metabolic abnormalities. Nusinersen is the first therapeutic approved for SMA. It is an antisense oligonucleotide to be administrated by intrathecal injection, that promotes SMN protein induction. Despite the efforts to identify the biomarkers of SMA progression and therapeutic efficacy, [2] little is known about how the metabolomic profile is conditioned by the disease and the treatments. We employed nuclear magnetic resonance (NMR) spectroscopy to longitudinally characterize the unknown metabolic effects of Nusinersen in the cerebrospinal fluid (CSF) of SMA patients across disease severity. The results revealed that modulation of amino acid metabolism is a common denominator of biochemical changes induced by Nusinersen, with distinct downstream metabolic effects according to disease severity. In severe SMA1 patients, Nusinersen stimulates energy-related glucose metabolism. In intermediate SMA2 patients, Nusinersen effects are also related to energy homeostasis but involve ketone bodies and fatty acid biosynthesis. In milder SMA3 patients, Nusinersen mainly modulates amino acid metabolism. These findings reveal disease severity-specific neurometabolic signatures of Nusinersen treatment, suggesting a selective modulation of peripheral organ metabolism by this CNS-directed therapy in severe SMA patients.[3]

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POSTER 32

DEVELOPMENT OF *N*-(1-ADAMANTYL)BENZAMIDES AS NOVEL ANTI-INFLAMMATORY MULTITARGET AGENTS ACTING AS CANNABINOID CB2 RECEPTOR AGONISTS AND FATTY ACID AMIDE HYDROLASE INHIBITORS

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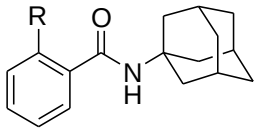
Cannabinoid subtype 2 receptor (CB2R), belonging to the endocannabinoid system, is overexpressed in inflammatory states and is involved in the onset of inflammation-based disorders as neurodegeneration and cancer. Recently, my research group identified three lead compounds (Figure 1), acting as dual drugs, able to simultanously activate CB2R and inhibit endocannabinoids degradation (through the inhibition of the enzyme Fatty acid amide hydrolase, FAAH, responsible for endocannabinoids degradation)[1]. With the aim to improve the pharmacodynamic profile of the three lead compounds 1,2,3, with also the aid of molecular docking simulation, structural modifications were performed that led to the identification of novel promising structural features for improved dual CB2R/FAAH activity.



Compound	R	CB2R, K_i, nM ± SEM	CB1R, K_i, nM ± SEM	FAAHi, IC_{50} (μM)
1	OBn	14.8 ±4.49	241.3 ± 2.4	4.0 ± 2.3
2	OPentyl	10.8 ± 1.9	152.9 ± 22	6.2 ± 1.4
3	OCH_2Cy	20.1 ± 3.7	67.6 ± 10.0	3.4 ± 0.4

Figure 1. Lead compounds.

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POSTER 33

FROM SIDE-CHAIN TO SIDE-CHAIN DISULFIDE TO SIDE-CHAIN TO TAIL MACROLACTAMBRIDGE FOR THE DEVELOPMENT OF POTENT CXCR4 PEPTIDE ANTAGONISTS

Mazzarella V.[a], Trotta A.M.[b], Tomassi S.[c], Ieranò C.[b], Merlino F.[c], Messere A.[a], D'Aniello A.[a], DelBene A.[a], Campagna E.[a], Mottola S.[a], Cosconati S.[*a], Scala S.[*b] and Di Maro S.[*a]

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As part of our ongoing efforts to develop novel CXCR4 antagonists as potential anticancer agents, we have previously reported the potent, selective, and plasma-stable peptide **3**.[1] However, the disulfide bond that has been extensively employed to constrain linear sequences showed some synthetic limitations, redox and metabolic instability. Here, we investigated the structural and biological effects ensuing from the disulfide bond replacement of peptide **3** with a side-chain to tail macrolactamization. The choice of this strategy was supported by our previous findings that **3** can tolerate different cyclization strategies to stabilize the overall conformation.[2] More importantly, this kind of cyclization could allow us to explore more in-depth the receptor region originally interacting with the C-terminalgroup of the L-Pen. This strategy produced two promising candidates (**13** and **17**) that displayed high affinity and selectivity against CXCR4. Moreover, more in-depth functional studies performed on CXCR4-overexpressing cancer cell lines provided evidence that both compounds were able to inhibit the CXCL12-mediated cell migration and increase the concentration of cAMP, which are well-validated hallmarks for the antagonistic activity. Encouraged by these results, molecular modeling studies are now ongoing on **13** and **17** to obtain a theoretical model that could serve as starting point for the synthesis of new analogs with higher affinity and stability compared to **3**. Finally, we believe that our outcomes could be translated to different GPCR-interacting peptides for the pursuit of novel chemical probes that could assist in dissecting the complex puzzle of this class of transmembrane receptors.

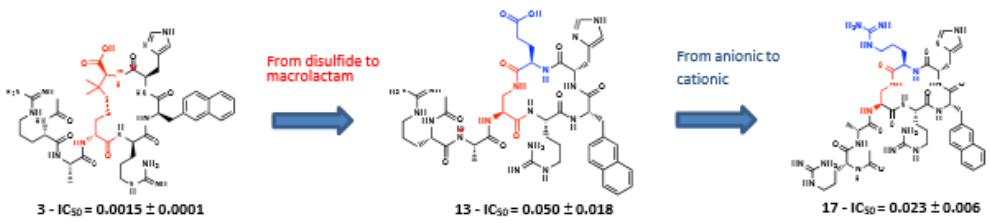

Figure 1. IC50 Values (Mean ± SD) for CXCR4 Antagonist Peptides Obtained Measuring the Inhibition of Receptor Binding of Anti-CXCR4 PE-Antibodies (Clone 12G5)

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POSTER 34

GUANIDINE-BASED CYCLIC ANTIMICROBIAL PEPTIDE DERIVATIVES OF TEMPORIN L

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Naturally occurring antimicrobial peptides (AMPs) offer promising solutions for combating antibiotic resistance by employing alternative mechanisms of action compared to conventional antibiotics.[1] Among these, Temporins derived from frog skin possess intriguing properties for biological research due to their: i) short sequence length, ii) effectiveness against a wide range of pathogens, iii) additional chemotactic and immunomodulatory effects.[2]

A notable isoform within this group is the 13-mer Temporin L (TL) peptide, which demonstrates high antimicrobial potency and a strong affinity for Gram-negative bacteria such as *P. aeruginosa* and *E. coli*. However, it is important to note that at microbicidal concentrations, TL also exhibits a significant level of hemolytic activity.[3] Based on our recent findings,[4] we have designed and synthesized new cyclic derivatives which bear a pendant or incorporated guanidino group in key positions of the TL peptide sequence. Given that higher concentration of guanidines is correlated with strong bacterial cell-lytic behavior and favors translocation across membranes,[5] this modification has been used to improve therapeutic index and 'drug-like' features of this class of AMPs. The preliminary results of antimicrobial activity and cytotoxicity obtained for this first set of guanylated TL derivatives will drive the development of further analogues whose bacterial membrane interaction properties and their potential against multi-drug resistant infectious diseases will be investigated.

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POSTER 35

CHEMICAL CHARACTERIZATION AND ANTICANCER ACTIVITY OF *LYCIUM BARBARUM* ON BREAST CANCER VIA ER STRESS ACTIVATION

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The endoplasmic reticulum (ER) plays a key role in the synthesis, folding, modification, and transport of proteins. An alteration in the homeostasis of ER, due to several factors such as redox imbalance, results in a condition called ER stress, characterized by an accumulation of damaged and not properly folded proteins in the lumen of the reticulum.[1] The compensatory response of cells to ER stress is known as unfolded protein response (UPR) and involves three signaling pathways: inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). They play vital roles in returning protein homeostasis to levels seen in non-stressed cells. However, if the level of stress is too high, UPR can activate apoptotic processes leading to cell death.[2, 3] In this regard, several natural compounds are able to modulate cell fate by inducing or reducing ER stress. For these reasons, the role of natural products targeting ER stress in the cancer is increasingly being investigated.[4, 5]
Lycium barbarum extract (LBE) contains a high content of bioactive polysaccharides and carotenoids with antitumour activity, but there is little current knowledge about the mechanism and active components of the extract on breast cancer cells.[6, 7] The aim of our study was the chemical and biochemical characterization of a LBE investigating whether its known components could have a pro-oxidant and consequent anticancer action on a human breast cancer cell line (MCF-7) through the modulation of UPR. LBE was investigated for its total phenolic, flavonoid, chlorophyll, carotenoids contents, as well as antioxidant activity detected by DPPH assay. More than 60 compounds belonging to different classes such as phenolic acids, phenylpropanoids and flavonoids, and hydroxy fatty acids have been identified by Ultra high-performance liquid chromatography–tandem mass spectrometry (UHPLC–HRMS/MS). Our findings demonstrate for the first time the ability of LBE to target UPR through an alteration of cellular oxidative stress, exerting an anticancer activity towards MCF-7 cell line.

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POSTER 36

ANTHOCYANINS: A PROMISING STRATEGY FOR THE MANAGEMENT OF OBESITY AND METAINFLAMMATION

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Currently, the health properties of natural polyphenolic compounds, particularly anthocyanins, with high antioxidant potential, have drawn attention to the management of obesity and related oxidative stress and metainflammation.[1] Based on our previous studies on bioactive polyphenols in cherries as a possible source of functional health foods, in this work three varieties of Apulian cherries (*Prunus avium* L., cv. Ferrovia, Sweetheart and Lapins) were evaluated and compared for extraction, characterization, and profiling of the antioxidant potential of polyphenolic compounds.[2,3] An eco-friendly extraction approach consisting in ultrasound-assisted extraction (UAE) was adopted and implemented with a factorial analysis design to improve extraction yields of polyphenols, particularly anthocyanins. The total phenolic content and antioxidant profile of cherry extracts were determined using Folin Ciocalteau, flavonoid, DPPH, and ABTS assays. HPLC-MS/MS analyses profiled 25 hydroxycinnamate derivatives and 17 flavonoids, including 4 flavan-3-ols, 8 flavonols and, especially, 5 anthocyanins (namely, cyanidin-3-O-sophoroside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, pelargonidin-3-O-glucoside, and petunidin-3-O-glucoside). Extracts of Lapins and Sweetheart, which were richer in polyphenols, returned the highest reducing power and radical scavenging capacity.

Although the scientific literature is sufficiently consistent to establish the modulation of oxidative stress pathways by anthocyanins in preventing or delaying obesity-related comorbidities, few results are available regarding the molecular targets of the bioactive compounds and the postulated mechanism of action. Emerging evidence emphasizes the role of adiponectin protein and its transmembrane receptors (AdipoR1 and AdipoR2) in signaling cascades involved in the obesity-associated pathway.[4] Inspired by this research topic and based upon our computational studies expertise, we examined *in silico* the mechanism by which cherry anthocyanins regulate adiponectin activity.[5] Our docking studies suggest that cherry anthocyanins may act as a ligand for AdipoR1 and AdipoR2 and potentially activate adiponectin. Overall, our results provide new insights into the benefits of anthocyanins in the prevention of obesity, including antioxidant and anti-inflammatory effects, and represent a valuable starting point for planning a research program aimed at both *in vitro* and *in vivo* screening of the antioxidant profile of anthocyanins from the fruit of Apulia cherries as a potentially useful bioactive resource for the management of obesity and understanding the molecular mechanism underlying obesity.

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POSTER 37

ANALYZING NICOTINE PROTECTION MECHANISM AGAINST AMYLOID TOXICITY IN NEUROBLASTOMA CELLS BY NMR-METABOLOMICS

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Pharmacometabolomics is emerging as an important tool for analyzing mode of action and toxicity mechanism of approved drugs and new molecular entities.[1] In this field, NMR showed a great potential since it's a non-destructive, highly reproducible, and quantitative method that requires a relatively easy sample preparation.[2]
Alzheimer's disease (AD) is a neurodegenerative disease characterized by the presence of amyloid plaques composed of Aβ peptides. Several small organic molecules demonstrated to prevent amyloid aggregation. Among them, nicotine has shown to slow down the aggregation of Aβ (1-42) in vitro by linking to the α-helix structure.[3,4] Since the mechanism of nicotine protection against amyloid toxicity is not still completely investigated, in this work we conducted an NMR metabolomics analysis on cell culture.
The effect of nicotine was monitored at different times in neuroblastoma cells SH-5YSY by analyzing the endo- and exo-metabolome, identifying the biochemical pathways involved and predicting differential genes expression.[5]
This work demonstrates that metabolomic analysis is a powerful approach to predict drug mechanism of action using a simple *in vitro* experimental model.

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POSTER 38

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTI SARS-CoV-2 AGENTS

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SARS-CoV-2 is the etiologic agent of Coronavirus Disease-19 (COVID-19), which from December 2019 has been responsible of an enormous health-socio-economic disaster. Vaccines development has given an important effect against the onset and the severity of the disease and have also contributed to the global economy recovery.[1] However the presence of vaccine-resistant variants has been reported and underlines the necessity of specific therapeutic agents for patients with severe COVID-19.[2]
In our previous work we described an interesting dual inhibitor acting against both virus proteases,[3] the main protease (M^{pro}) and papain-like protease (PL^{pro}) involved in the viral polyproteins cleavage (IC_{50} versus M^{pro} = 1.72 μM; IC_{50} versus PL^{pro} = 0.67 μM).[4]
Starting from these promising results, in this work we present the design of a second library of small molecules retaining the indolic scaffold with the aim to increase the antiviral activity. All compounds were screened for their enzymatic activity and for their ability to reduce virus growth in different SARS-CoV-2 variants infecting Vero cells. Many derivatives showed a considerable efficacy in both experiments, differently, some of them exhibited an inconsistent enzymatic efficacy despite their high cellular activity. Thus, further investigations are still ongoing to assess their direct interference with further targets.

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POSTER 39

GLUCOLINES: GLUCAMINE-QUINOLINE CONJUGATES WITH ANTINEURODEGENERATIVE PROPERTIES

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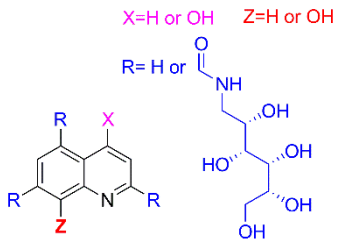
Alzheimer's disease (AD) is a neurodegenerative disease exhibiting significant intricacy in its pathology involving several pathogenic features, such as proteopathy, metal ion dyshomeostasis, and oxidative stress. Mounting evidence indicates the independent and synergistic contributions of amyloid-beta (Aβ), related to the protheopatic implications, metal ions, and oxidative stress toward neurodegeneration.[1] Quinoline (Q) derivatives have shown their ability to target one or more aspects of AD.[2] Herein, we report the synthesis and biological evaluation of a new class of compounds, glucolines (Scheme 1), obtained conjugating glucamine with Qs. Glucamine was chosen because it was hypothesized that its properties similar to glycosides and the ability to form supramolecular adducts with lipophilic organic compounds could impart antiaggregant properties and water-solubility to the Q derivative. To evaluate the potential of glucolines as multitarget-directed ligands, the newly synthesized compounds were tested for their interaction toward Aβ, Cu ions, the Cu–Aβ system also in the presence of Zn ions, and as antioxidant agents. The findings confirm that the new molecules can target and modulate several pathological features of AD.



Scheme 1. Schematical structure of Glucolines

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The author thanks SELECTION and 3N-ORACLE projects.

POSTER 40

SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTICANCER PRODRUGS BASED ON COPPER-BINDING COMPOUNDS

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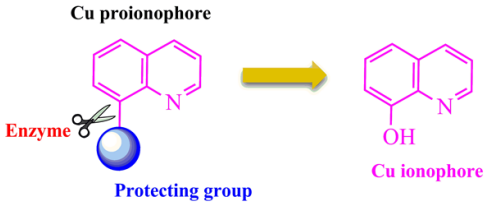
Cancer therapies suffer from severe off-target effects because most of them target critical aspects of cells that are generally shared by all rapidly proliferating cells. The development of new effective therapies should aim to reduce side effects, increasing the selectivity of the administrated drugs.[1] In addition, these agents should overcome cancer cell resistance and target cancer stem cells. Some copper ionophores have shown promise in this direction thanks to an intrinsic selectivity in preferentially inducing cuproptosis of cancer cells compared to normal cells.[1,2] Cuproptosis defines Cu-dependent cytotoxicity with a unique mechanism leading to cell death.[3] In this context, we synthesized a series of systems that could act as prodrugs. In particular, proionophores are molecules that have to be activated to release the metal ionophore, increasing the selectivity of the drug. In particular, stimulus-responsive prodrugs of 8-substituted quinolines were evaluated in vitro. The released quinoline moiety acts increasing the copper content of cancer cells and causing cell death.



Scheme 1. Schematics of Copper proionophores

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POSTER 41

DESIGN OF PPARS/FXR MODULATORS FOR THE TREATMENT OF LIVER DISEASES BY GENERATIVE MODELS

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Metabolic syndrome, a cluster of conditions including obesity, type 2 diabetes mellitus, and dyslipidemia, is widely recognized as a major risk factor for cardiovascular diseases (CVDs). It also affects the liver, leading to non-alcoholic fatty liver disease (NAFLD) and its severe form, nonalcoholic steatohepatitis (NASH).[1] Peroxisome proliferator-activated receptors (PPARs) and Farnesoid X receptor (FXR) are nuclear receptors that play crucial roles in regulating various metabolic processes. The development of dual modulators targeting PPARs and FXR has gained significant attention due to the potential to improve therapeutic outcomes for NASH and type 2 diabetes mellitus.[2] In this project we aim to identify novel compounds able to modulate PPARs/FXR by integrating virtual screening with deep learning techniques. [3-5] In particular, we employed generative models, which belong to a specific category of deep learning algorithms.[6] The generated molecules will be acquired, if commercially available, or proposed for synthesis and then evaluated in biological assays, e.g., transactivation assays, expression of a set of target genes regulated by PPAR/FXR and cellular metabolism analyses. By applying several selection criteria, we assembled a curated dataset from ChEMBL, made up of SMILES strings. This dataset was subsequently fed into the model, a Conditional Variational Autoencoder (CVAE), allowing the acquisition of the chemical rules for molecule production. The metrics employed to evaluate the generated molecules encompass uniqueness, validity, novelty, quantitative estimate of drug-likeness (QED) and synthetic accessibility score (SAS).
In view of the prospective model fine-tuning phase, we then collected a set of active ligands that specifically target the nuclear receptors PPARs and FXR. In this step, only compounds showing activity values (K_i and IC_{50}, as detailed above) of at least 10 µM were selected. The proposed approach represents a valuable tool for *de novo* molecule design and has the potential to accelerate the molecular optimization cycle time.

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POSTER 42

NMR STUDIES OF THE REACTION ENVIRONMENT OF THE PHOTO-MICELLAR CATALYSED SYNTHESIS OF AMIDES FROM ISOCYANIDES

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The amide bond is one of the most important linkages in biological molecules and is present in 25% of marketed drugs.[1] Recently, some visible-light-mediated synthetic methods have been reported to achieve amide bond formation,[2-4] and in particular, the ability of isocyanides to act as geminal radical acceptors to yield amides was demonstrated by Rohe et al. Indeed, photoredox catalysis is emerging as a powerful tool to exploit visible-light-absorbing photocatalysts, which upon electron or energy transfer processes are able to sensitize organic molecules and trigger a photochemical reaction.[5-8] Moreover, the merging of micellar and photoredox catalysis represents a key issue to promote "in water" photochemical transformations, thus drawing synthetic organic chemistry procedures closer to Nature's mild reaction conditions.[9-11] In this context, we developed an NMR based protocol to study the reaction environment at the atomic level of a photo-micellar catalyzed synthesis of amides from isocyanides. In particular, we studied the localization of the photocatalyst $[Ir(ppy)_2bpy]PF_6$ relative to the surface and the interior of two micellar systems SDS or CTAC which turned out to be the most and the least efficient reaction systems, respectively. The study was carried out determining the NOE contacts between photocatalyst and the micelles and using specific paramagnetic probes, 16-doxylstearic acid (16-doxyl) and Mn^{2+}, for the position determination. NMR analysis demonstrated that the catalyst is on average positioned on the micelle surface and can flip from the outer to inner part of it in the case of SDS while it is deeply inserted in CTAC micelles. The obtained experimental data allow a rational approach for selecting the best reaction conditions suggesting that, for an optimal catalytic efficiency, the photocatalyst must be positioned on the micelles' surface and almost free to move inside and outside the micelles. We are confident that the rationalization of the optimum photocatalyst/ surfactant pairing could drive the exploration and the optimization of future photomicellar-catalyzed reactions, thus promoting the merging of these intrinsically green chemical approaches underlying the flourishing of in water photoredox catalytic transformations.

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POSTER 43

switchSENSE Y-STRUCTURE TECHNOLOGY: CHARACTERIZATION OF TERNARY COMPLEX FORMATION OF PROTACs™ FOR THE LYSINE METHYLTRANSFERSE G9a

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PROteolysis TArgeting Chimeras (PROTACs™) are heterobifunctional molecules designed to divert the degradation of proteins of interest (POIs) by engaging the ubiquitin-proteasome system.[1] Despite the emerging role of these degraders as promising tools in drug discovery, their characterization requires an array of additional systems compared to conventional small molecule inhibitors, making their development challenging.[2]

Herein we report the application and the subsequent optimization of switchSENSE heliX+ technology to study and characterize ternary complex formation, giving unique insights into the affinity (K_D) and kinetics (k_{on}, k_{off}) of binding. This biophysical and biosensor assay consists of a dsDNA Y-structure combining a pair of donor-acceptor fluorochromes (green and red dyes) hybridized with the POI and E3 ligase (Figure 1). In detail, we characterized the PROTACs™ ternary complex between the E3 ligases, CRBN and VHL, and the epigenetic protein target G9a, a lysine methyltransferase responsible for histone H3 lysine 9 (H3K9) mono – and dimethylation.[3] The simultaneous binding of the PROTACs™ to G9a and VHL or CRBN brings together the green donor and the red acceptor dye closer and thereby induces red fluorescence emission due to Förster Resonance Energy Transfer (FRET).

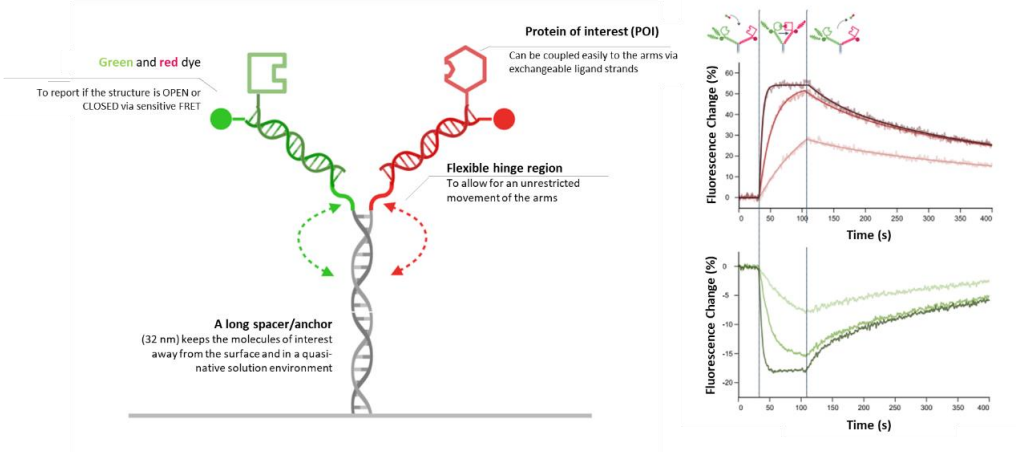

Figure 1: Y-shaped DNA nanostructures bestowed with a fluorochrome FRET-pair to screen PROTACs™.

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POSTER 44

HARMALINE SCAFFOLD AS ALLOSTERIC ACTIVATORS OF THE MITOCHONDRIAL PROTEASE Clp P: A CHALLENGE TO DEVELOP A TREATMENT FOR THE PEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMA (DIPGp)

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Pediatric diffuse intrinsic pontine glioma (DIPGp) is a rare aggressive tumor that grows diffusely in the pons. Because of its infiltrative nature and inoperability, DIPGp is the leading cause of brain tumor death in children. Despite advances achieved in new neurosurgical techniques and numerous clinical trials helpful in identifying new chemotherapeutic agents, prognosis remains poor. Approximately 600 children die every year from DIPG worldwide. The current standard treatment used in DIPGp is focal noncurative radiation therapy, which often leads only to transient improvement in neurological function.[1] ONC201, a known dopamine D2 receptor (DRD2) antagonist, represents the only useful drug for the treatment of DIPGp for which there is much hope although not always effective. ONC201 is being approved for phase III clinical trial for children and adults with low- and high-grade gliomas, including DIPGp. In 2019, through diffractometry (X-rays), the biological target of ONC201 was identified as the human caseinolytic protease P (*h*ClpP), a mitochondrial serine protease that is allosterically activated by ONC201, resulting in an alteration of mitochondrial homeostasis that triggers cell death. In this context, a wide program aimed at identifying scaffolds to drug repositioning was initiated to find out new *h*ClpP activators that could overcome the pharmacokinetic and pharmacodynamic limitations of ONC201. Structure Based Virtual Screening study by Fingerprints for Ligands and Proteins (FLAP) identified harmaline among a big set of natural compounds. Harmaline is a member of the β-carboline class, and by preliminary *in vitro* testing seems a promising activator of *h*ClpP.

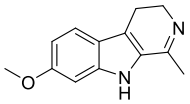

Figure 1. Chemical structure of harmaline.

Harmaline is a natural fluorescent alkaloid with a tricyclic pyrido[3,4-b]indole ring structure (Figure 1), which has been widely studied for its broad spectrum of action in the treatment of a variety of pathological conditions. Modifications of harmaline chemical structure is ongoing for Structure-Activity Relationship investigation as an attempt to maximize *h*ClpP activity.

Acknowledgement

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POSTER 45

STRUCTURE-ACTIVITY RELATIONSHIPS OF 3,5-DIHYDROBENZO[E][1,4]THIAZEPIN-2(1H)-ONE DERIVATIVES AS NCX3 ENHANCERS

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The relationship between Ca^{2+} dysregulation and neuronal death has been well-documented in several neurodegenerative diseases, in this regard a relevant role is played by several ionic transporters, including the sodium-calcium exchanger (NCX). Starting from the neuroprotective profile of CGP37157,[1] known as mNCX blocker, and SAR studies on NCX modulators,[2] we have designed several pharmacomodulation of the 3,5-dihydrobenzo[e][1,4]thiazepin-2(1H)-one nucleus in order to improve the selectivity and the potency. The scaffold, variously substituted, has been linked in position 1 to a cyclic amine via an acetyl spacer. To investigate the interaction and explore the effect of stereoisomery on the affinity towards the selected target, we used the 2-methylpiperidine as racemic mixture and the enantiomerically pure forms. Compounds **1**-**12** have been screened on NCX3 isoform activity by an HTS approach on BHK cells singly expressing this isoform. The increased NCX3 activity in *reverse* mode, or the inhibition, was evaluated by measuring the Na^+-free-dependent Ca^{2+} level above the mean of basal value. The compounds were also functionally characterized by patch-clamp electrophysiology and Fura-2AM video imaging. In this way, we have identified twelve pharmacological modulators of NCX3, able to change the calcium currents in *reverse* and *forward* mode. Notably, the 7-chloro-substituted compounds showed a clear enhancing activity compared with the 7-nitro derivatives. Among them, the (S)-2-methylpiperidine-substituted diastereoisomers strongly enhanced the *reverse* and *forward* modes of operation of NCX3, instead the compounds (R)-2-methylpiperidine-substituted were less active or even inhibitors. In the final round of the SAR study, a chiral phase separation of the compound **12** was carried out obtaining two pure enantiomers, **S1** and **S2**, assessed to the same pharmacological evaluation. In the light of the data, it might assume that the stereochemistry of the 2-methylpiperidine linked to the N_1 it's crucial for the biological activity of these molecules. In addition, the C-5 chirality could be decisive to achieve a difference in forward and reverse operation mode. Future studies will include the determination of absolute configuration of each enantiomers and Computer-Aided SAR analysis.

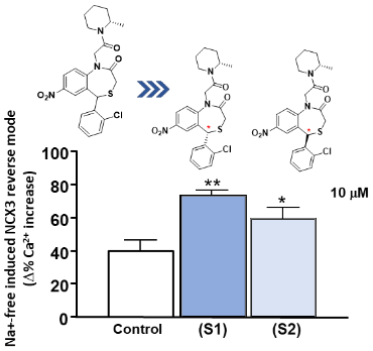

Figure 1. HTS of the enantiomers S1 and S2

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POSTER 46

"DUAL ANTA-INHIBITORS" OF THE A_{2A} ADENOSINE RECEPTOR AND CASEIN KINASE CK1DELTA: SYNTHESIS, BIOLOGICAL EVALUATION, AND MOLECULAR MODELING STUDIES

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By screening an in-house chemical library of A_{2A} adenosine receptor (AR) antagonists, endowed with an adenine structure and tested as CK1δ inhibitors, the 2-chloro-9-cyclopentyladenine was selected for further modifications. Hence, a series of 9-cyclopentyladenine derivatives substituted at the 2/N^6 and 2/8-positions were synthesized and evaluated for their ability to inhibit the CK1δ enzyme and to bind ARs. Such molecules were obtained starting from the commercially available 2,6 dichloropurine that was converted in the desired compound in 4, 7 or 10 synthetic step depending on which positions were introduced the appropriate substituents. Binding studies[1] at ARs and inhibitory assay at CK1δ enzyme demonstrated that the N^6-acetonitrile-2-chloro-cyclopentyladenine (IC_{50} = 0.36 μM) resulted in the most active CK1δ inhibitor of the series, while the 2-chloro-8-thiophenyl-9-cyclopentyladenine (KiA_1 = 0.053 μM and KiA_3 = 0.017 μM) and the 2-chloro-8- furyl-9-cyclopentyladenine (KiA_{2A} = 0.007 μM) showed the best affinity at the A_1/A_3 and A_{2A} ARs. Although the most potent enzyme inhibitor showed a moderate affinity for ARs and vice versa, some compounds, that we called "dual anta-inhibitors, were found to possess enzyme inhibitory activities with IC_{50} values in the sub-μM range and AR affinities with nM Kis. Hence, the 2-chloro-N^6-(2-phenylethyl)-9-cyclopentyladenine (KiA_3 = 0.151 μM, IC_{50} = 0.66 μM) and the 2-chloro- N^6-(3-phenylpropyl)-9-cyclopentyladenine (KiA_1 = 0.692 μM, IC_{50} = 0.73 μM) resulted in dual anta-inhibitors of CK1δ and A_1 and A_3 ARs, respectively, while the 9- cyclopentyl-2-dimethylaminoadenine (KiA_{2A} = 0.123 μM, IC_{50} = 1.75 μM) and 9-cyclopentyl-2-dimethylamino- N^6-methyl-(2-benzimidazolyl)adenine (KiA_{2A} = 0.076 μM, IC_{50} = 0.59 μM) were dual anta-inhibitors of CK1δ and A_{2A}ARs. Computational studies were performed to simulate, at the molecular level, the protein–ligand interactions involving all the compounds of our series. The 9-cyclopentyl-2-dimethylamino-N6-methyl-(2-benzimidazolyl)adenine, endowed with the best balance of the two activities, represents the first ever reported "dual anta-inhibitor" of the A_{2A}AR and CK1δ enzyme, and is the leading compound of potential therapeutic agents with synergistic neuroprotective effects.

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POSTER 47

QUICKEN THE CURE FOR LAFORA DISEASE: A DOCKING-BASED DRUG REPURPOSING STUDY AGAINST HUMAN GLYCOGEN SYNTHASE

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Lafora disease (LD) is a rare neurodegenerative disease whose clinical symptoms arise in the early adolescence: such signs – including seizures, blindness, visual hallucinations – rapidly tend to worsen over years. This rare disease belongs to glycogen storage diseases (GSDs) and it is due to loss-of-function mutations of genes involved in glycogen metabolism, which results altered: precipitates of poorly branched, hyperphosphorylated glycogen – which accumulate into so-called Lafora bodies (LB) – are diagnostic characteristic of this pathology.[1] The failure of treatment by anti-seizure drugs and the lack of a defined pharmacological therapy aggravate this dramatic scenario. In this perspective, research goal-oriented to speed up the drug discovery process, such as the application of the drug-repurposing strategy within the Structure-Based Virtual Screening (SB-VS) approach, becomes highly significant. Considering that medicines already approved for commerce has yet regarded as safe drugs by regulatory authorities, the application of drug-repurposing strategy within the computer-aided drug design can rapidly steer toward a pharmacological therapy for LD with lower overall development costs and shorter development timelines. A database of marketed drugs, equipped with their Anatomic Therapeutic and Chemical (ATC) code, has been tested against the catalytic site of human glycogen synthase, a potential pharmacological target of LD.[2] The SB-VS has unveiled the group of drugs acting on musculo-skeletal system as high-affinity medicines which can be reprofiled as drugs potentially useful for LD treatment by reducing the glycogen accumulation in the brain.

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POSTER 48

AN INTEGRATED MACHINE LEARNING MODEL TO SPOT PEPTIDE BINDING POCKETS IN 3D PROTEIN SCREENING

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Peptide-protein interaction (PePI) systems are utmost importance to tackle the onset of severe diseases, including cancer and neurodegenerative pathologies. The spread of artificial intelligence and machine learning likely changed the perspectives into PePI systems research although the identification of putative druggable peptide interacting regions represents still an open challenge. In this work, we present an innovative machine learning model based on Linear Discriminant Analysis (LDA) demonstrating to be highly predictive in detecting putative protein binding regions of small peptides (Figure 1) [1].

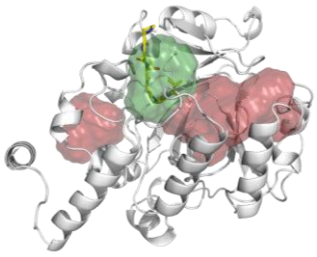

Figure 1. Example of putative pockets in peptide-protein complex

Starting from a collection of 439 high-quality pockets derived from peptide-protein crystallographic complexes (PixelDB database), three sets of well-established peptide-binding regions were firstly selected through a Partitioning Around Medoids (PAM) clustering algorithm based on morphological and energetic 3D GRID-MIF molecular descriptors. Next, LDA-based protocol implemented in BioGPS [2] automatically detected the best combination between all the putative interacting peptide cavities and related GRID-MIF scores. This integrated classification model proved successfully to distinguish actual interacting peptide cavities from the rest of the protein surface (AUC = 0.86 and early ROC enrichment at first 5% equal to 0.48). Finally, LDA-based model has been successfully challenged on two external benchmarks of crystallographic peptide-protein complexes (i.e., InterPep and Pepsite) proving to be effective to the peptide drug discovery processes, including 3D protein virtual screening campaigns.

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POSTER 49

TIRESIA: AN ARTIFICIAL INTELLIGENCE-BASED WEB PLATFORM TO INVESTIGATE DEVELOPMENTAL TOXICITY OF CHEMICALS

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TIRESIA (Toxicology Intelligence and Regulatory Evaluations for Scientific and Industry Applications)[1] is a novel and easy-to-run digital platform for *in silico* assessment of Developmental Toxicity, one of the most relevant toxicological human health endpoints. TIRESIA implements an optimized Explainable AI-based algorithm which has been recently published by using the established CAESAR[2] training set made of 234 chemicals with experimental toxicological annotations.

TIRESIA has been validated on two test sets, including as a whole 585 chemicals, with performances comparable with other parallel approaches.

Users can interrogate TIRESIA by simply drawing the chemical structure of a given query or, alternatively, by pasting its SMILES (Simplified Molecular Input Lines Entry System) notation returning results in a standard report in portable document format, with additional details concerning the model applicability domain and SHAP explainability analyses[3]. Noteworthy, the output is equipped with a meaningful explainability support system that reveals which key chemical descriptors are relevant to address the interpretability of results.

We are confident that the proposed study could offer the opportunity to correctly investigate and predict developmental toxicity in the process of drug discovery, both at academic and industrial levels.

TIRESIA is available free of charge at http://prometheus.farmacia.uniba.it/tiresia/.

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POSTER 50

SOLVENT MIXTURES FOR GREEN SOLID PHASE PEPTIDE SYNTHESIS

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Solid phase peptide synthesis (SPPS) is the preferred technique for the synthesis of bioactive peptide. In recent years, because of environmental pollution, more interest is being given to green chemistry applicable to pharmaceutical companies and academies. Unfortunately, SPPS cannot be considered green because of the large amount of waste generated throughout the process. In addition, the most commonly used solvents are N, N-dimethylformamide (DMF) and dichloromethane (DCM) which are unsafe for humans and the environment.[1] As the green SPPS is a field still little explored, the objective of this work is to study solvents that reflect some of the twelve principles of green chemistry and that are applicable to all the steps of the SPPS.[2] Some solvents, such as p-cymene, can come from renewable sources (plants, biomass) and are therefore considered environmentally sustainable. Unfortunately, many of these solvents have different physical chemical properties than DMF. To avoid this problem, solvent mixtures are used.[3] Solvent mixtures have been used in the phases of swelling, deprotection, coupling and washing of SPPS obtaining good results.

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POSTER 51

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW BENZIMIDAZOLE-BASED INHIBITORS OF PD-L1

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Histone post-translational modifications (PTMs) 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, responsible of 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,[2] whose overexpression perturbs the cell cycle, induces chromosome instability, and leads to tumorigenesis,[3-5] 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.[6] 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), in-cell click-proteolysis targeting chimeras (CLIPTACs) and Hydrophobic Tags (HyTs), paving the way for future progress in Spindlin1 biology and its therapeutic applications (Figure 1).

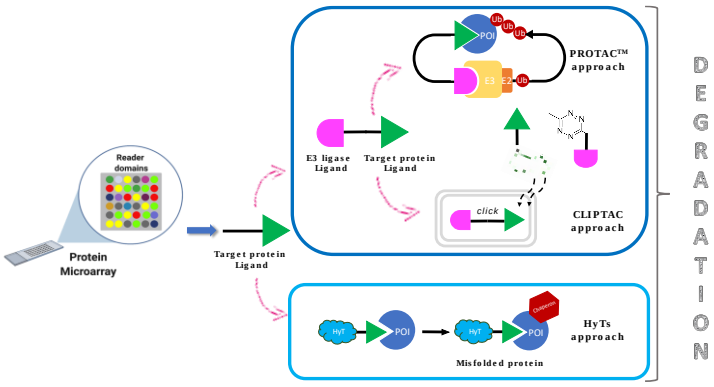

Figure 1. Aim of the work

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POSTER 52

CIRCE: A MULTI-LAYER FRAMEWORK FOR THE CLASSIFICATION OF SELECTIVE AND UNSELECTIVE CANNABINOID BINDERS BASED ON EXPLAINABLE MACHINE LEARNING

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The endocannabinoid system, which includes CB1 and CB2 receptor isoforms is tangled in the onset of several conditions including neurodegeneration, cancer, neuropathic and inflammatory pain, obesity, and inflammatory bowel disease. Given the high sequence homology between CB1 and CB2 receptor isoforms, understanding what are the molecular motifs for finding selective ligands is very challenging. In the study presented herein, a multi-layer machine learning framework has been employed to predict compounds with selective and unselective CB1/CB2 activity[1]. For model crafting CB1/CB2 compound activity data was fetched from the ChEMBL database (release 31)[2] and the core-substituent molecular fingerprint was used as descriptor[3]. Finally, Shapley values[4] were computed to identify features determining correct predictions and explain machine learning models. The newly developed models are expected to better support the identification of new active compounds for CB1 and/or CB2 or the repurposing of known drugs.

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POSTER 53

ANALYZING NICOTINE PROTECTION MECHANISM AGAINST AMYLOID TOXICITY IN NEUROBLASTOMA CELLS BY NMR-METABOLOMICS

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Pharmacometabolomics is emerging as an important tool for analyzing mode of action and toxicity mechanism of approved drugs and new molecular entities.[1] In this field, NMR showed a great potential since it's a non-destructive, highly reproducible, and quantitative method that requires a relatively easy sample preparation.[2]
Alzheimer's disease (AD) is a neurodegenerative disease characterized by the presence of amyloid plaques composed of Aβ peptides. Several small organic molecules demonstrated to prevent amyloid aggregation. Among them, nicotine has shown to slow down the aggregation of Aβ (1-42) in vitro by linking to the α-helix structure.[3,4] Since the mechanism of nicotine protection against amyloid toxicity is not still completely investigated, in this work we conducted an NMR metabolomics analysis on cell culture.
The effect of Aβ(1–42) on cells in presence and in absence of nicotine was monitored at different times in neuroblastoma cells SH-5YSY by analyzing the endo- and exo-metabolome, identifying the biochemical pathways involved and predicting differential genes expression.[5]
This work demonstrates that metabolomic analysis is a powerful approach to predict drug mechanism of action using a simple *in vitro* experimental model.

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POSTER 54

PROTEOCHEMOMETRIC MODELING OF NON-ZINC BINDING MMP-2 INHIBITORS

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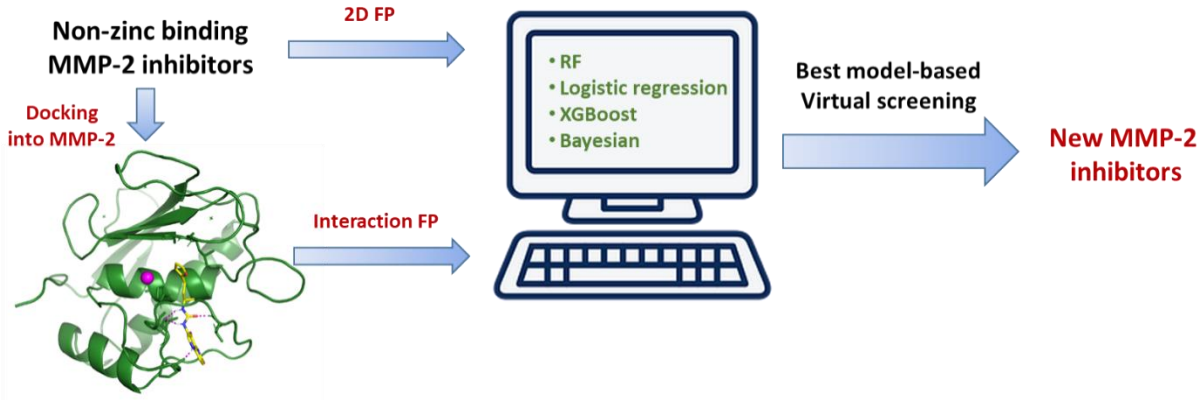
MMP-2, one of the most studied members of the MMP family, is a zinc-dependent endopeptidase involved in several pathologies, such as cancer, chronic inflammation, neurodegeneration, and cardiovascular diseases.[1,2] Despite the large interest this target has received for decades, no clinical compounds have been disclosed because of selectivity issues.[3] In our research path in this field, we identified several putative non-zinc binding MMP-2 inhibitors bearing different scaffolds endowed with micromolar activity. Docking studies suggest that the inhibition mechanism can be ascribed to selective contacts in the S1' site where ligands can exploit interaction with the selectivity loop. In the pursuit of our studies, we exploited the previously acquired knowledge about non-zinc binding MMP-2 inhibitors to find new ligands. This challenging task prompted us to apply a proteochemometric approach and combine information from both the inhibitors and the target to extract compound properties effectively related to biological activity. Several machine learning algorithms were tested and evaluated based on their ability to elicit the ligand and ligand-protein interaction fingerprints responsible for the MMP-2 inhibition not involving the zinc ion. The best-performing model was exploited for further virtual screening of a library of drug-like compounds. The enzyme inhibition assay on selected compounds allowed the identification of a new inhibitor endowed with micromolar activity and a new scaffold. Analogue testing confirmed the scaffold's validity and suitability for further studies.



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POSTER 55

SYNTHESIS AND VIRTUAL SCREENING OF NATURE-INSPIRED PHOSPHODIESTERASE 9 LIGANDS

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Dementia is a syndrome that represents a public health issue worldwide, given its high incidence. It is the result of several conditions, such as Alzheimer's disease (AD) and vascular dementia (VaD), which manifest themselves in the form of memory loss, disorientation, and progressive cognitive decline.[1] To date, many mechanisms have been reported in literature to be involved in pathophysiological processes, which makes research to solve dementia challenging. Among all the pathways, the one involving cyclic adenosine 3',5'-monophosphate (cAMP), cyclic guanosine 3',5'-monophosphate (cGMP), and the respective cellular effectors like phosphodiesterases (PDEs) are extensively studied.[2,3]

There are eleven PDE families (PDE1 to PDE11) which hydrolyze cAMP and/or cGMP with a different degree of selectivity and produce different results.[4] PDE9 is expressed in brain and is constituted by a catalytic domain formed by 16 helices that associate as dimers. Of note, two residues (Gln453 and Phe456) and metal ions (zinc and magnesium) important for ligand-target interaction are conserved among PDEs families and are required for influencing the activity of the enzyme.[5]

Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs that allow further modifications, since isopropenyl moiety and hydroxyl and/or carboxyl groups that can be easily functionalized.[6] In this context, betulin, betulinic acid (BA), and their derivatives have been demonstrated to have a variety of biological activities, including antioxidant, neuroprotective and anti-inflammatory roles as well as PDE inhibitory properties.[7,8,9]

In this work, we focused on PDE9 since it is overexpressed in the brain of patients affected by dementia.[10,11] More specifically, we reported the preparation of an analogue obtained through derivatization of BA which could represent a good candidate for obtaining new potential ligands.

The first phase of the work consisted in the synthesis of the compound, obtained by derivatization of BA with 1*H*-benzo[*d*][1,2,3]triazol-1-ol. Then, full characterization was performed by NMR, high-resolution mass spectrometry (HRMS), infrared (IR) and ultraviolet spectroscopy (UV).

Subsequently, interactions with the target(s) and the ADME profile of the molecule were computed and studied by means of computational tools.

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POSTER 56

NEW BLOOD-BRAIN BARRIER PERMEABLE COMPOUNDS AS NAEGLERIA FOWLERI CYP51 INHIBITORS

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Primary amoebic meningoencephalitis (PAM) is a fatal disease with 97% fatal rate outcome caused by free-living ameboflagellate *Naegleria fowleri* present in soil and warm fresh water. Upon accidental introduction into the nose, the ameba invades the central nervous system which leads the death of the infected patients within a week. Despite been stated as a rare disease with only 381 global cases, these cases could be the tip of an iceberg considering the dramatic loss of patience and complexity of its diagnosis.[1,2] Additionally, there is no standard treatment and patients are reported to be treated with Amphotericin B either alone or in combination with other drugs. However, its toxicity and side effects such as acute infusion-related reactions and dose-related nephrbotoxicity makes it difficult for its clinical use.[3,4] Overall, these facts indicate PAM as an unmet medical need. Moreover, sterol 14-demethylase (CYP51) has been reported as an essential drug target in literature and there are studies indicating the use of CYP51 inhibitors such as antifungal conazole drugs accompanied with Amphotericin B despite their low blood-brain barrier (BBB) penetrance in PAM cases.[5]

Within this work, we would like to highlight the potential use of miconazole-like compounds for PAM treatment. By testing 124 compounds *in silico* against *N. fowleri* trophozoites, we have obtained 9 hits having $EC_{50} \leq 10$ µM, which was further identified *via* cross - co-crystallization with the NfCYP51 target, leading to identification of the best drug-target for miconazole-like scaffolds. Accordingly, a set of analogs was synthesized and biochemically evaluated. These results showed excelled superiority of having S- over R-configuration and the advantage of ether over ester linkage at the scaffolds. Moreover, the two most promising structures, having improved EC_{50} and K_D were further tested for their brain penetrance. The result of brain-to-plasma distribution coefficient highlights a potential for further optimization of the compound as a drug candidate.

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POSTER 57

Q-RAKTION: A SEMIAUTOMATED KNIME WORKFLOW FOR BIOACTIVITY DATA POINTS CURATION

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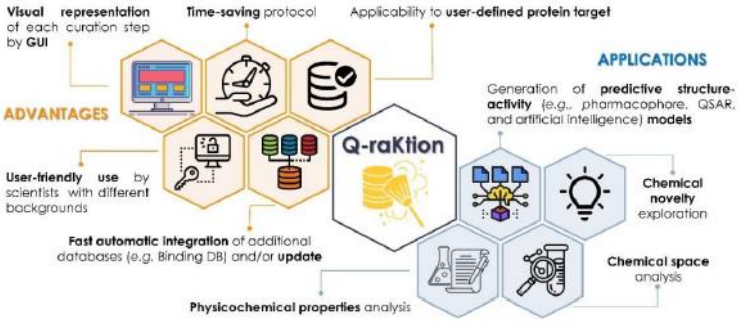
Predictive models can be developed using a wealth of ready-to-use data that has recently become more readily available to the scientific community and stored as activity datapoints in chemogenomic repositories. However, the lack of uniformity and consistency of data from various sources, which necessitates a process of integration and harmonization, strongly offsets the advantages provided by the availability of such a vast amount of accessible information.1,2 While various automated pipelines for processing and evaluating chemical data have been developed over the past few years, the curation of bioactivity datapoints is a less explored area with helpful concepts offered but no practical tools available. In this context, the present work represents a step towards filling this gap by providing a tool to meet the needs of end-users in building proprietary high-quality datasets for further studies. Specifically, we herein describe Q-raKtion,3 a systematic, semi-automated, flexible, and customizable KNIME workflow that effectively aggregates information on biological activities of compounds retrieved by two of the most comprehensive and widely used repositories, namely PubChem and ChEMBL.

The main advantages of the developed tool consist of i) the availability of a general pipeline that integrates different data curation and integration tasks, ii) the use of a graphical interface that allows the visual representation of each curation step, and iii) the high propensity of the protocol to be easily interpretable and reproducible. Overall, Q-raKtion facilitates access to bioactivity data curation for scientists with different backgrounds, enabling the building of proprietary high-quality datasets for the specific needs of a research project. The pipeline is developed within KNIME, a user-friendly open-source platform that ensures a flexible and customizable module organization with other successful applications. Of note, the Q-raKtion tool can potentially be applied to create high-quality dataset of molecules that modulate any target of interest to the user.

As a practically case study, we reported the application of the Q-raKtion pipeline to build a well-annotated, high-quality dataset of AKT1 serine/threonine kinase inhibitors.



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POSTER 58

TRIGGERING RNA DEGRADATION WITH SMALL MOLECULES: DEVELOPMENT OF RIBONUCLEASE TARGETING CHIMERAS (RIBOTACs)

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RNAs represent a class of biological molecules that are involved in a variety of cellular functions. There are many non-coding RNAs (ncRNAs) beyond the well-known role of RNA in protein synthesis machinery, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which are known to play a role in cancer, and viral and bacterial infections.[1-3] For this reason, ncRNAs have become an innovative source of targets for medicinal chemistry campaigns. We can now develop different strategies to tackle diseases by targeting RNA because of the advancements in RNA structure characterization and the growing disclosement of the RNA targeting principles *via* small molecules. As part PNRR's funding, this project aims to develop ribonuclease targeting chimeras (RIBOTACs), which are multifunctional molecules that trigger the degradation of ncRNAs of interest through the enzymatic degradation by RNase L nuclease.[4] We will target miRNA21, a short ncRNA whose dysregulation is often observed in human diseases, including solid tumors like bone cancer and triple-negative breast cancers.[5] In this context, we will respectively use the fragment-based and ligand-based medicinal chemistry approaches to design a series of warheads against miRNA21 secondary structures. Subsequently, we will combine the warheads with RNase L recruitment module, spaced by PEG linkers of different lenghts to obtain the corresponding RIBOTAC molecules. (Figure 1) After a thorough characterization of the specific RIBOTAC-miRNA21 interactions at the molecolar level, we will evaluate the actual target engagment in cell systems to validate and further optimize the designed RIBOTACs.

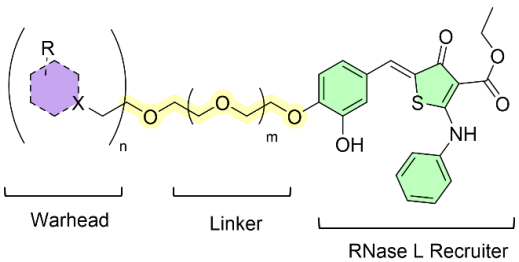

Figure 1. Schematic representation of a RIBOTAC, which includes a generic structure representative of the warhead (purple), different-length PEG linkers (yellow) and the RNase L recruiter unit (green).

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POSTER 59

DOCKING-BASED DESIGN OF NOVEL INHIBITORS TARGETING MRP1 IN CANCER DRUG DISCOVERY

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Breast and ovarian cancers are significant threats to women's health, with millions of cases diagnosed annually. In this study, the Life Chemicals chemoinformatics team focused on finding new drug-like compounds that could target the multidrug resistance-associated protein 1 (MRP1), which is associated with drug resistance [1]. MRP1 is overexpressed in cancer cells in the ovaries and mammary glands, preventing the entry of drugs from the basolateral side and leading to resistance toward available drug classes [2].

First, we optimized the Life Chemicals high-throughput screening (HTS) Compound Collection on the basis of the Ligprep module and OPLS3e force field for drug discovery against MRP1. Then, four possible sites on the protein surface were detected, using the SiteMap's algorithm (Fig. 1). *In silico* docking was performed on the 2CBZ protein structure utilizing the Schrödinger software package. ADME predictions involving the QikProp of the Schrödinger software package enabled us to identify 990 promising compounds with excellent bioavailability and non-toxicity. Thus, target screening of these compounds can open up the way for the development of safe, oral anticancer drugs in the future.

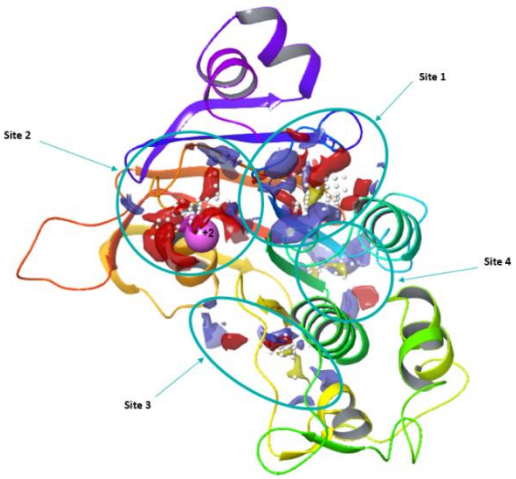

Figure 1. Prediction of sites on the surface of the MRP1 protein.

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POSTER 60

DEVELOPMENT OF 1,3,4-OXADIAZOLE-SUBSTITUTED 3-BR-ISOXAZOLINES AS POTENT ANTI-TRYPANOSOMAL AGENTS

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Human African Trypanosomiasis (HAT), also known as "sleeping sickness", is a life-threatening vector-borne disease caused by *Trypanosoma brucei* (*T. brucei*) parasites. It mostly affects poor rural populations in sub-Saharan Africa. However, climate change is predicted to impact the transmission dynamics of vector-borne diseases, shifting the disease burden. The existing HAT treatment options are old, toxic, and drug resistance is becoming a major challenge. Phenotypic screening approaches have been successfully used for the discovery of new antiparasitic compounds, as highlighted by the recent approval of fexinidazole for the treatment of HAT. Phenotypic screenings allow the rapid identification of compounds active against the parasites. These approaches enable the swift selection of relevant molecules because the proof of biological activity considers membrane permeability, cell uptake and efflux mechanisms. Using a cell-based phenotypic assay, we screened a series of known antimalarial and antileishmanial agents[1], and identified a potent anti-*T. brucei* molecule (**2**, Fig. 1) bearing a 1,3,4-oxadizole core linked to a 3-bromo-4,5-dihydroisoxazole (B*DHI*) ring. The 1,3,4-oxadiazole is a privileged scaffold in antiparasitic drug discovery, while the BDHI core is a moderately reactive, drug-like warhead able to react with activated cysteines leading to irreversible inactivation of the target protein. The presence of the BDHI moiety can also be exploited for proteomic studies aimed at target(s) identification. We performed Structure-Activity Relationship (SAR) studies around the lipophilic tail of compound **2** and synthesized a library of derivatives. The novel molecules were tested against T. brucei parasites, and screening against mammalian cells (THP-1) was employed as a counter-screen to filter out cytotoxic compounds. The cyclopropyl-substituted molecule displayed a high potency compared to the parental compound, together with an improved selectivity. The intrinsic reactivity of the BDHI warhead was evaluated at different pH using glutathione and N-acetyl cysteine, through a LC-MS method. The compounds were also submitted to early ADME-toxicology profiling to guide hit selection. Overall, our results pave the way for the development of novel agents to fight HAT.

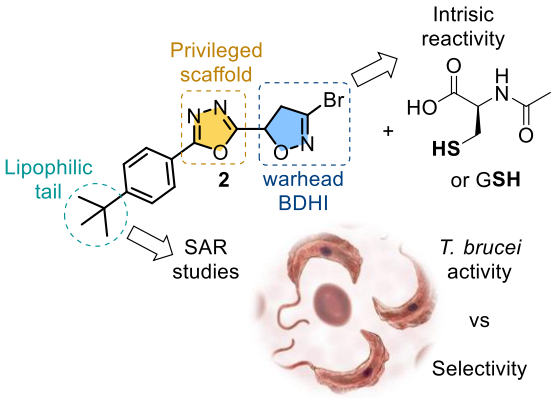

Figure 1

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POSTER 61

NOVEL STERILE ALPHA AND TOLL INTERLEUKIN RECEPTOR MOTIF CONTAINING-1 (SARM1) INHIBITORS AS POTENTIAL NEUROPROTECTIVE AGENTS

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The coenzyme nicotinamide adenine dinucleotide (NAD) plays an important role in energetic metabolism in the cells. Since NAD is consumed by the cellular processes, it needs to be continuously replenished. Its biosynthesis is ensured by multiple biosynthetic pathways, mainly *de novo*, the Preiss-Handler, and the salvage pathways, which occur in different combinations depending on the cell type and metabolic status.[1,2]

The axon non-apoptotic death process, termed Wallerian degeneration (WD), is common to a large number of neurological disorders and human diseases, such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ASL), peripheral neuropathies, etc. Two NAD-related enzymes with a mandatory activity to the process, are nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) and sterile alpha and toll interleukin receptor motif containing-1 (SARM1), a Toll-like receptor adaptor protein of multidomain architecture.[3]

SARM1 acts downstream of NMNAT2, and its early and selective activation is required before degeneration can start or develop, through its capacity to consume NAD and generate the second messenger cADPR, which promotes cell-autonomous axonal self-destruction. Therefore, SARM1 inhibition is recognized as a broad strategy to block neuropathies.

Some SARM1 inhibitors, such as isoquinolines, isothiazoles, nicotinamide, and nicotinic acid mononucleotide (NaMN, an NAD+ precursor) have been identified so far.[4] However, to better understand the complex mechanism of SARM1 regulation, more potent inhibitors are needed.

In this work, the synthesis and biological evaluation of novel potential SARM1 inhibitors will be reported.

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POSTER 62

OLIVE MILL WASTEWATER AS A RENEWABLE SOURCE OF NUTRACEUTICALS USEFUL FOR BIOMEDICAL APPLICATION

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Extra virgin olive oil (EVOO) is a widely studied health-promoting food, presenting nutraceutical properties mainly attributed to its unique composition, and in particular to polyphenols. EVOO production generates huge amounts of related wastes such as olive mill wastewaters (OMWWs) and olive leaves, which have a great impact in term of sustainability and environment due to their difficult management and disposal.[1] On the other hand, several studies described olive leaves and OMWWs as source of bioactive compounds with nutraceutical properties, including antioxidant, anti-inflammatory and antimicrobial effects.[2,3] Recently, our research group reported the design of new nutraceutical biodevices composed by biocompatible polyhydroxyalkanoate fibers (PHAs) incorporating olive leaf extract (OLE). The new OLE/PHA fibers presented anti-inflammatory and immunomodulatory properties, essential in wound healing and tissue regeneration.[4,5] Starting from these promising results, the purpose of this interdisciplinary project is the further valorisation of EVOO by-products through the development of new valuable biodevices useful in different biomedical fields, in which OMWWs were incorporated in specific PHA biocompatible nanofibers (Figure 1). This project agreed with the objectives of the Recovery and Resilience Plan (PNRR) in terms of environmental sustainability, in particular concerning the valorisation of food supply chain waste, which significantly affects the environmental impact.

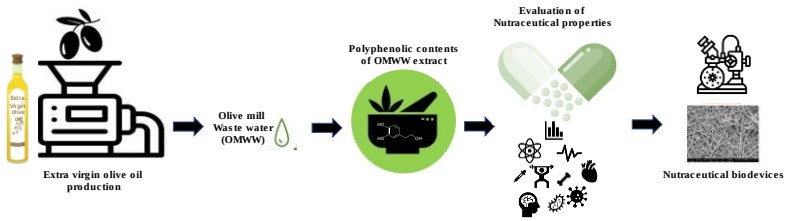

Figure 1

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POSTER 63

BENZOIMIDAZOTRIAZINEDIONE: A PROMISING SCAFFOLD FOR ALDH1A SELECTIVE INHIBITORS AS ANTICANCER AGENTS

Bernardoni, B.L.;[a] Castelli, G.;[a] Petrarolo, G.;[a] Siragusa, S.;[b] Garavaglia, S.;[b] Mori, M.;[c] La Motta, C.;[a] and D'Agostino, I.[a]

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Cancer is estimated to account for tens of millions of deaths per year. Despite several efforts in developing new therapeutic strategies, the raising drug-resistance phenomena along with the delay in diagnosis and treatment due to the COVID-19 pandemic worsened the expectancy of patients. A big concern is related to cancer stem cells (CSCs), being able to evade traditional anticancer treatments and establish a recurrent tumor or metastasis. In this context, a growing interest in Aldehyde Dehydrogenase (ALDH) enzymes has been recorded. ALDHs (EC: 1.2.1.5) are NADP-dependent oxidoreductases playing crucial roles in the maintenance and differentiation of CSCs, thus, their overexpression or dysfunction has been demonstrated to promote chemoresistance and survival of this cell population.[1] Although multi-ALDH isoform inhibitors have been proposed, poor bioavailability and high aspecificity/toxicity limited their development, shining the spotlight on the more attractive isoform-selective ALDH1A inhibitors. The past acquired expertise in benzoimidazotriazinedione (BITD, **Figure 1**) core synthesis of our research laboratories[2] and its structural similarities with isatin (**Figure 1**), a compound largely exploited for the obtainment of effective ALDH1A inhibitor,[3] drove us to perform a computational-aided design of BITD derivatives. Through a microwave-assisted 3-step approach, a focused library of 2,10-disubstituted BITDs was synthesized. Their preliminary enzymatic investigation confirmed a preference for ALDH1A1 inhibition, laying the foundation for a further development.

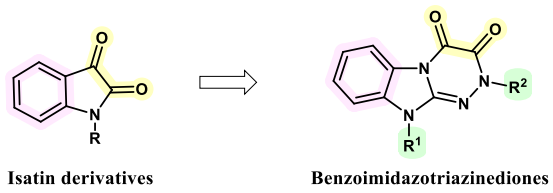

Figure 1. Structures of Isatin and BITD derivatives in this study.

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POSTER 64

SULFONIUM ANALOGS OF THE SILENT AGONIST NS6740 TO TARGET THE ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR

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NS6740 is an archetypal alpha7 nicotinic acetylcholine receptor (nAChR) silent agonist. It produces a small receptor activation when applied alone while inducing a long-lived receptor desensitization that responds to the type II positive allosteric modulator PNU-120596.[1,2] NS6740 evidenced promising anti-inflammatory activity by reducing the microglia release of tumor necrosis factor-α stimulated by lipopolysaccharide exposure,[3] and showed antinociceptive effects in mouse models of peripheral neuropathy and tonic inflammatory pain.[4] The SAR analyses of NS6740 revealed that the protonatable nitrogen atom is essential to its peculiar silent activity profile.[1] In this study, we substituted sulfur for nitrogen and generated a set of new NS6740-related sulfonium congeners (Figure 1).[5] These new derivatives represent an alternative way to design ligands for the nAChR that provides different structural and electronic features than traditional nitrogen-containing ligands and may lead to unique means of targeting unwanted processes such as inflammation. The synthetic approach to the new sulfonium analogs and the electrophysiological data obtained on human alpha7 nAChRs expressed in *Xenopus laevis* oocytes with two-electrode voltage clamping technique will be presented and discussed. The results of computational calculations and docking simulations will also be illustrated to highlight the molecular interactions of the sulfonium ligands within the orthosteric binding site of the cryo-EM structure of the alpha7 receptor.

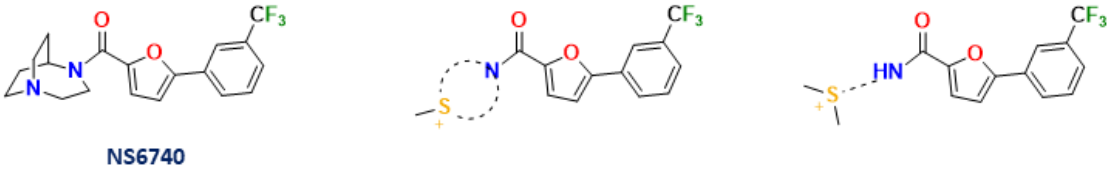

Figure 1. Chemical structure of the lead compound NS6740 and general structures of the sulfonium analogs.

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POSTER 65

LIPIDOMICS STUDY OF NEUROBLASTOMA CELLS UNDER Aβ$_{1-42}$ TOXIC INSULT

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Lipids in neuronal membranes play important roles in cell signaling. Accordingly, changes in brain lipids that occur in Alzheimer's disease (AD) have attracted scientific interest to give insight at the neurodegenerative mechanism.[123] Indeed, in the present work we aimed to explore the possible connections existing between amyloid aggregation and cell lipid alterations, both primary events in AD pathogenesis.[4] LC-HR-MS was used in a lipidomic approach to highlight lipid variations in connection with amyloid toxicity on differentiated human neuroblastoma derived SH-SY5Y cells. Then, with an open outcome, the study was focused to find out some new lipid-based biomarkers that could result from the interaction of amyloid peptide with cell membrane and could justify neuroblastoma cells neurotoxicity.[5]

Hence, differentiated SH-SY5Y cells were treated with increasing concentration of Aβ$_{1-42}$ peptide for different incubation times, mimicking the different aggregation states of the peptide. Lipids were extracted after cell cryo-lysis by homogenizer using single-phase 2-propanol-water (90:10 v/v). The LC-MS analysis of samples was performed by a RP-UHPLC system coupled with a quadrupole-time-of-flight mass spectrometer in a comprehensive data-independent SWATH acquisition mode. Data processing was achieved by MS-DIAL (version 4.24).

Each lipid class profile in SH-SY5Y cells treated with Aß$_{1-42}$ was compared to the one obtained for the untreated cells to identify and relatively quantify some altered species in various lipid classes. This approach was able to shed light on some peculiar lipid alterations that might be related to different Aß$_{1-42}$ aggregation species and to explore the cellular response mechanisms to the toxic stimuli. The obtained results coincide with the ones in literature by lipidomic analysis on cerebrospinal fluid and plasma of AD patients. Our research will continue by unveiling the effect of amyloid aggregation inhibitors on the lipid profile of neuroblastoma cells.

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POSTER 66

^{64}Cu PHOSPHINO COMPLEXES AS NEW POTENTIAL DIAGNOSTIC DRUGS FOR PET/CT IMAGING

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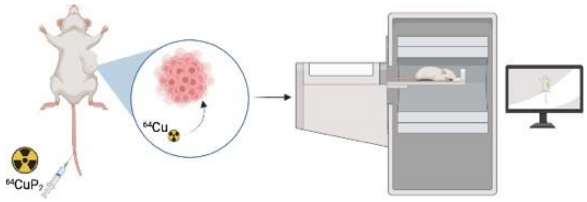
Theragnostics is an emerging treatment strategy that combines therapeutics with diagnostics. It associates both a diagnostic investigation that identifies patients most likely to be treated with a targeted drug therapy based on the diagnostic results. In this field, radiotheragnostics is one of the most clinically advanced application of theragnostic in oncology and positron emission tomography (PET) imaging is the most used investigation technique[1]. Imaging not only is non-invasive and can be repeated during therapy, but also evaluates the entire tumor giving important information about tumor heterogeneity. Although images recorded by using ^{18}F radiolabelled compounds account for most PET scans, there is currently significant interest in the development of new PET radiotracers based on the coordination of metallic radionuclides[2]. Among all, ^{64}Cu have been extensively studied and the interest towards this isotope has increased after the FDA approval of Copper (^{64}Cu) oxodotreotide in2020[3]. Copper-based complexes have been originally investigated as potential anticancer agents on the assumption that endogenousmetals may be less toxic than non-essential metals for normal cells.Moreover, copper plays an important role in some mechanisms involved in the abnormal cell proliferation, over all angiogenesis.



The higher necessity of copper in cancer cells is provided by an over-expression of the trans-membrane copper transporter protein hCTR1 which is selective for monovalent cations[4]. Among all, several Cu(I) phosphino complexes have shown encouraging perspective in both in vitro and in vivo studies. Here we report the radiosynthesis and the characterization of two homoleptic Cu(I) complexes with two phosphino ligands (^{64}CuP$_2$, P = tertiary phosphine), previously investigated for their anticancer activity, using the radionuclide ^{64}Cu. In addition, uptake studies on a panel of aggressive KRAS mutant pancreatic cell lines as well as PET/CT imaging (Figure 1) and biodistribution studies in human xenografts models have been performed.

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POSTER 67

FIRST-IN-CLASS DUAL NON-ATP COMPETITIVE GLYCOGEN SYNTHASE KINASE 3β/HISTONE DEACETYLASES INHIBITORS AS POTENTIAL THERAPEUTIC TO TREAT ALZHEIMER'S DISEASE

De Simone, A.;[a] Santini, A.;[b] Tassinari, E.;[b] Loi, M.;[c] Trazzi, S; Ciani, E.;[c] Poeta, E.;[d] Monti, B.;[d] Daniele, S.;[e] Piccarducci, R.;[e] Martini, C.;[e] Pruccoli, L.;[b] Tarozzi, A.;[b] Bersani, M.;[a] Spyrakis, F.;[a] Andrisano, V.;[b] and Milelli, A.[b]

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One of the main goals of an Alzheimer's Disease (AD) therapy is to restore the perturbated networks characterizing the pathology. In light of this, the application of Multitarget drugs (MTDs) able to modulate more than one target involved in the onset of the disease represents a successful strategy. However, there is a clear consensus that the chance of developing a successful MTDs increases if it is directed, among the possible, "networked" targets[1]. Following this principle, in 2019 we have reported the first-in-class MTDs able to target Glycogen Synthase Kinase 3β (GSK-3β) and Histone Deacetylases (HDACs)[2]. These two targets are deeply involved in AD's pathogenesis. The selection of GSK-3β and HDACs was based not only on their specific roles in the neurodegenerative process, mainly related to the hyperphosphorylation of tau protein and the regulation of cognitive process, respectively, but also on their engagement in the same networks i.e. tau phosphorylation[3]. Based on the above considerations and with the aim of developing a second generation of dual inhibitors, we report the design, synthesis, and preliminary biological evaluations of the first set of dual non-ATP competitive GSK-3β/HDACs inhibitors[4]. To design dual GSK-3β/HDACs inhibitors, we sought to combine in a single chemical entity the pharmacophoric groups responsible for binding to GSK-3β, in a non-ATP competitive manner, and HDACs. We, thus, performed a Structure-Activity Relationships (SAR) and obtained compound **1**, selected for further biological investigation.

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POSTER 68

THE MULTICOMPONENT PASSERINI REACTION IN THE DIVERSITY-ORIENTED DEVELOPMENT OF SOFT AND HARD TRPV1/CB MODULATORS

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The well-known functional crosstalk between cannabinoid receptors CB1 and CB2 and the transient receptor potential vanilloid type 1 (TRPV1) offers promising polypharmacological strategies to treat CNS-related disorders, inflammatory processes and pain.[1] Even though the differential structural mechanism involved in TRPV1 activation by endocannabinoids (*e.g.*, anandamide (AEA), *N*-arachidonoyl dopamine (NADA), and *N*-oleoyldopamine (OLDA)), compared with the classic agonist capsaicin, has been elucidated, a few dual TRPV1/CB modulators have been identified so far.[1,2] Worrisome systemic and local adverse effects led to the termination of several drug development programs focused on a number of TRPV1 agonists and antagonists, suggesting the need for safer strategies.[3] In this context, the soft drug (SD) approach[4] was successfully applied by us to develop capsaicinoid-based soft modulators endowed with a good potency at TRPV1 and controlled hydrolytic stability, as well as *in vivo* anti-nociceptive activity.[5] On the basis of these encouraging outcomes,[5,6] we exploited the multicomponent Passerini reaction to access high chemical diversity in the preparation of libraries of α-acyloxy carboxamides as soft and hard drugs.[7] By combining the polar headgroups of AEA, NADA and OLDA, as well as of other CB2/TRPV1 ligands and CB2 inverse agonists[8] with a pool of fatty acids, we discovered micromolar TRPV1 agonists and antagonists endowed with balanced CB agonism. As expected, metabolic stability studies in systems expressing esterase activity (*e.g.*, HaCaT homogenates, human plasma, skin and liver S9 fractions), as well as in both primary keratinocytes and fibroblasts, showed different susceptivity to hydrolysis of soft and hard TRPV1/CB modulators. For the most promising soft dual agents identified, biological evaluation of the *N*-substituted 2-hydroxyacetamide metabolites did not show any activity at the main targets, in line with the SD principle. Of note, well-balanced lead compounds endowed with drug-like properties have been selected and are being evaluated as topical and systemic agents in *in vivo* models of pain and inflammatory skin disorders.

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POSTER 69

STYRYL-THIAZOLE HYBRIDS AS NOVEL ANTI-ALZHEIMER DRUG CANDIDATES

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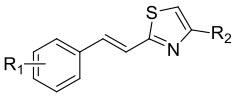
Herein, combining the thiazole and cinnamoyl groups into the styryl-thiazole scaffold, a series of novel styryl-thiazole hybrids (6a-p) was rationally designed, synthesized, and evaluated by the multi-target-directed ligands strategy as potential candidates for the treatment of Alzheimer's Disease (AD).[1,2] Hybrids 6e and 6i were the most promising among the synthesized hybrids since they were able to significantly increase cell viabilities in $A\beta_{1-42}$-exposed-human neuroblastoma cell line (6i at the concentration of 50 µg/mL and 6e at the concentration of 25 µg/mL resulted in ~34% and ~30% increase in cell viabilities, respectively). Compounds 6e and 6i exhibited highly AChE inhibitory properties in the experimental AD model at 375.6 ± 18.425 mU/mL and 397.6 ± 32.152 mU/mL, respectively. Moreover, these data were also confirmed by docking studies. Compared to hybrid 6e and according to the results, 6i also has the highest potential against $A\beta_{1-42}$ aggregation with over 80% preventive activity. The in-silico prediction of the physicochemical properties of hybrids confirmed that 6i possesses a better profile compared to 6e. Therefore, compound 6i presented a promising multi-targeted active molecular profile for treating AD considering the multifactorial nature of AD, and it is reasonable to deepen its mechanisms of action in an *in vivo* experimental model of AD.



6a: R_1=H, R_2=H
6b: R_1=H, R_2=OH
6c: R_1=H, R_2=OMe
6d: R_1=H, R_2=NO_2
6e: R_1=H, R_2=Br
6f: R_1=4-Me, R_2=OH
6g: R_1=4-Me, R_2=OMe
6h: R_1=4-OMe, R_2=OH
6i: R_1=4-OMe, R_2=OMe
6j: R_1=3,4-OMe, R_2=OH
6k: R_1=3,4-OMe, R_2=OMe
6l: R_1=3,4,5-OMe, R_2=OH
6m: R_1=3,4,5-OMe, R_2=OMe
6n: R_1=Cl, R_2=OH
6o: R_1=F, R_2=OH
6p: R_1=NO_2, R_2=OH

Figure 1. Chemical structure of 6a-p.

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POSTER 70

DETECTION AND QUANTIFICATION OF DRUG AND ANTI-DRUG ANTIBODIES IN PATIENTS TREATED WITH THE THERAPEUTIC MONOCLONAL ANTIBODY ADALIMUMAB USING A SURFACE PLASMON RESONANCE BIOSENSOR

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Adalimumab (ADL) is a Tumor Necrosis Factor α (TNF-α)-blocking monoclonal antibody approved for the treatment of several autoimmune diseases, *e.g.*, Non-infectious Uveitis, Juvenil Idiopathic Arthritis, and Rheumatoid Arthritis. Despite ADL is a fully human IgG1-type antibody, the development of anti-ADL antibodies (ADAbs) is reported in treated patients at a rate up of 8% after 8 weeks and 24% after 60 weeks. The presence of ADAbs is supposed to be linked to treatment failure and adverse effects, such as hypersensitivity reactions.[1,2] Although different studies demonstrated a correlation between ADAbs presence and higher disease activity, their direct comparison is challenging due to different ADAbs detection assays used.[3,4] In fact, the methodologies employed probably break down at the lower end of the detectable range and consequently the development of more sensitive drug-tolerant assay is fundamental to evaluate the correlations between ADAbs and disease outcome, in order to personalize the therapy depending on each patient response to the treatment and leading to benefits both for patient's health and at the financial level.[5]

Herein we propose a method to measure and quantify independently ADL and ADAbs using a Surface Plasmon Resonance (SPR)-based optical biosensor. This technique allows to measure in real-time mass variations caused by the binding of the analyte flowing onto a biochip surface bearing an immobilized ligand. Particularly, Adalimumab immobilization on the chip surface allowed the quantification of ADAbs directly in patients' sera, without any particular sample preparation procedure. Moreover, the immobilization of an anti-ADL monoclonal antibody made it possible to directly detect and quantify the free ADL in sera samples, another important parameter in the evaluation of the therapeutic treatment efficacy. The methods were optimized to overcome relevant problem due to the presence of non-specific binding of the sera samples, which was addressed using an irrelevant antibody as blank, thus completely removing the non-specific signal without affecting the specific response. Other main issue to be solved was the correct chip regeneration after each analysis, in order to reuse the biosensor for a large number of samples. This important step was accomplished through a first injection at low pH, followed by two injections at high pH.

The optimized methods for ADL and ADAbs were successfully validated, testing positive and negative control sera previously characterized using a commercial ELISA kit. The data obtained show a good agreement between ELISA and SPR in both Adalimumab and anti-Adalimumab antibodies detection and quantification, opening the door to a possible routine use of SPR-based biosensor for disease monitoring in clinical practise.

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POSTER 71

COMPUTATIONAL AND BIOLOGICAL STUDIES FOR THE IDENTIFICATION OF NEW RHODOPSIN-STABILIZING COMPOUNDS

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Rhodopsin, a G-protein coupled receptor (GPCR) protein found in the retina's rod cells, has two main components: an opsin protein and a light-sensitive molecule called *11-cis retinal*. When light enters the eye, the phototransduction mechanism starts: *11-cis* retinal isomerizes to *all-trans*-retinal, causing a conformational change in the opsin and triggering a series of biochemical reactions that eventually lead to the generation of electrical signals that are transmitted and processed by the brain to generate a visual perception (Figure 1). Mutations in *RHO*, the gene that codes for rhodopsin, can lead to various eye disorders, such as retinitis pigmentosa (RP), which causes progressive retina degeneration, leading to vision loss and, eventually, blindness. Some *RHO* mutations lead to the death of rod cells by causing opsin misfolding or reducing its stability. In vitro studies suggest that small retinoid-based chaperones can partially rescue the correct folding and trafficking of P23H *RHO*, a mutant form of the rhodopsin protein. However, these chaperones have limitations due to photoinduced isomerization that can occur during protein synthesis in the endoplasmic reticulum, which can cause protein instability.[2] This work used a computational approach to search for new non-retinoid compounds that stabilize wild-type rhodopsin (wt). To identify new compounds, a receptor-based virtual screen (VS) study employing pharmacophore modelling, consensus docking, and molecular dynamics (MD) simulations was carried out to discover new rhodopsin-stabilizing non-retinoid compounds. The compounds identified by the virtual screening have been purchased and tested on wt rhodopsin, reconstituted from pig opsin using the 9-cis retinal isomer. These preliminary biological studies suggest that some compounds identified with VS bind rhodopsin, compete with 9-cis retinal, and reduce rhodopsin regeneration with an effect similar to that of beta-ionone, a known binder to this receptor. The potential stabilizing effect will also be evaluated in the mutated rhodopsin for its impact on misfolding, which represents the real targets of a potential pharmacological approach to the RP.

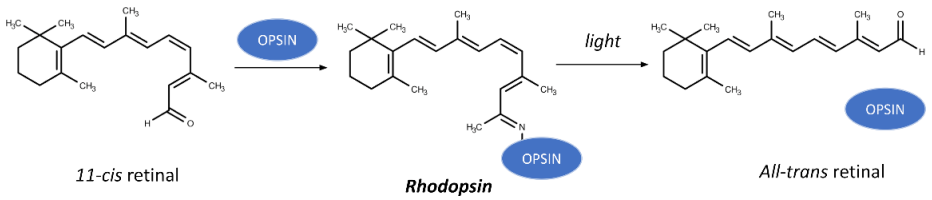

Figure 1. Mechanism of action of phototransduction activation.

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POSTER 72

PURSUING THE PROTAC APPROACH TO COMBAT EMERGING FLAVIVIRUS INFECTIONS

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Flaviviruses are vector-borne RNA viruses causing severe diseases, including epidemics of dengue and West Nile Virus (WNV), and the recent outbreak of Zika virus.[1] Measure to control and cure flavivirus-induced infections are largely underestimated. To mitigate current health burden and limit future outbreaks, drugmakers need to be ready and avoid that the spread of flaviviruses will materialize, in the worst-case scenario, in a pandemic. Currently, there is neither vaccine nor drug to treat flavivirus infection, making drug-discovery efforts extremely urgent. Despite different families of small molecules or peptidomimetic inhibitors have been reported, none of them reached clinical phases.[2] Here, as game-changer, we are proposing the proteolysis targeting chimeras (PROTAC) approach to address *Flavivirus* infections through the targeting of the NS2B-NS3 protease.[3] PROTAC are heterobifunctional molecules with the ability to harness ubiquitination and subsequent proteasomal degradation, by putting in close proximity the protein of interest (POI) and an E3 ligase. In fact, PROTAC are structurally constituted of a ligand, directed to the POI, bridged to an E3 ligase by a suitable linker (Figure 1). [4] The NS2B-NS3 protease is essential for the replication of flaviviruses and, importantly, is conserved among WNV, Dengue and Zika viruses.[5] Therefore, our final PROTACs should possess pan-flaviviral activity and might represent a new avenue to target flaviviruses.

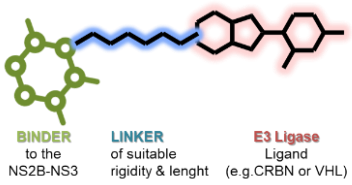

Figure 1. Schematic representation of a PROTAC

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POSTER 73

NEW FAK INHIBITORS INDUCING IMMUNOGENIC CELL DEATH

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Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase overexpressed in several solid tumors, which plays a crucial role in the regulation of adhesion, cell migration, and the cell cycle. Additionally, FAK acts as an immune modulator across cancer types. Recently, we described the synthesis and the antiproliferative activity of a series of 3-(imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)-1*H*-indole derivatives, which showed potent *in vitro* antiproliferative and antimigratory activity against numerous cancer cell lines belonging to the NCI full panel and PDAC panel, with GI_{50} values ranging from micromolar to sub-micromolar level.[1] Further studies identified the inhibition of phosphorylation of PTK2/FAK as the mechanism of action of this class of compounds. Based on the interesting antiproliferative activity observed for the imidazothiadiazole scaffold and the FAK inhibitory activity described in literature for pyridine compounds[2] we synthesized new analogs bearing 3-pyridinyl and 4-pyridinyl nucleus at position 6 of the imidazothiadiazole nucleus, with the aim to obtain new anticancer agents targeting FAK.

Interestingly, some of the synthesized compounds exhibited an outstanding antiproliferative activity against several human cancer cell lines, being able to induce cancer immunogenic cell death (ICD). These data confirm the potential of the imidazothiadiazole nucleus as promising scaffold for the development of new anticancer agents.

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POSTER 74

TARGETING G-QUADRUPLEX STRUCTURES: SYNTHESIS AND BIOPHYSICAL STUDIES OF NEW GUANYLHYDRAZONE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

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Among innovative targets for anti-cancer research, non-canonical structures of nucleic acids like G-quadruplex (G4), have represented an interesting focus in medicinal chemistry with the design and synthesis of new G4-ligands. These binders would interact with G4 structures and stabilize them, leading to the blocking of the catalytic activity of enzymes such as DNA, RNA polymerases and telomerase, and eventually to cell death. A diimidazopyrimidine core[1] was first described as a starting point: the use of SAR and biological assays results, together with techniques like FRET and CD spectroscopy, were helpful to optimize the design, the activity and selectivity of the synthetized analogues affording the synthesis of two molecules (**FG**, **FIM**, **Figure 1**),[2] considered as lead compounds for new G4 binders. The less cytotoxic derivatives were also able to induce IFN-B production,[3,4] useful to contrast cancer development. Furthermore, inspired by the positive results obtained from our previously synthetized compound **1** (**Figure 1**),[5] we built a small new library with new guanylhydrazone ligands bearing different heterocyclic cores like imidazothiazoles or indole scaffolds, that were tested in their interaction with nucleic acids.

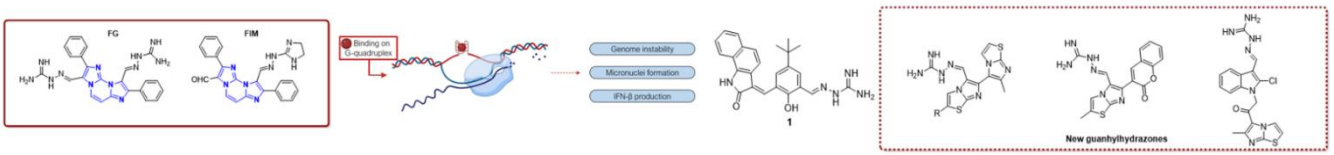

Figure 1.

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17th-20th September 2023 / Chieti, University "G. d'Annunzio" of Chieti-Pescara

POSTER 75

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 4-AMINO-PYRAZOLO[3,4-*d*]PYRIMIDINESAS POTENTIAL SRC KINASE INHIBITORS

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The non-receptor tyrosine kinase (TK) Src plays a pivotal role in the signal transduction pathways involved incell proliferation, survival, migration and angiogenesis.[1] Src is implicated in different pathologies, including cancer, due to its strong proto-oncogenic activity,[2] representing a key target for designing new therapeutic antitumor agents. Therefore, Src small molecule inhibitors belonging to different chemical classes have beenreported.[3]

In this context, we focused on the synthesis of a large library of pyrazolo[3,4-*d*]pyrimidine derivatives activeas Src inhibitors, allowing a wide-ranging structure-activity relationship (SAR) evaluation.

In particular, compound **SI306** (**Figure 1**) exhibited good activity on different cancer cell lines overexpressingSrc, i.e., SHSY-5Y neuroblastoma and U87 glioblastoma cell lines, and also in *in vivo* models of these tumors.[4,5]A **SI306** prodrug was synthesized to overcome the poor aqueous solubility of SI306, improving *in vitro* and *invivo* activity.[6] Furthermore, a SAR analysis pointed out that the introduction of a bromine atom on the *para* position of the N1 side chain phenyl ring afforded compounds endowed with improved potency towards theT315I Bcr-Abl mutant and in a few cases against Src.[7] Starting from these data, we decided to synthesize a new small library of 4-amino-pyrazolo[3,4-*d*]pyrimidines (**CMPs1**) bearing a bromine atom on the *para* position of the N1 phenyl side chain and a thioethylmorpholine chain on C6, analogously to SI306 (**Figure 1**).In particular, we applied a rational design study to our library of pyrazolo[3,4-*d*]pyrimidines, combining the most potent Src inhibitors bearing a bromine atom on the N1 side chain (**CMPs2**) with the most potent Src inhibitors bearing the thioethylmorpholino substituent on C6 (**CMPs3**) (**Figure 1**).

Moreover, a virtual list of *para* bromo analogues of in-house Src inhibitors (K_i < 30 nM) was designed and docked into the catalytic pocket of Src to avoid losing any new potential Src inhibitors. The synthesis of new compounds and preliminary biological results will be discussed.

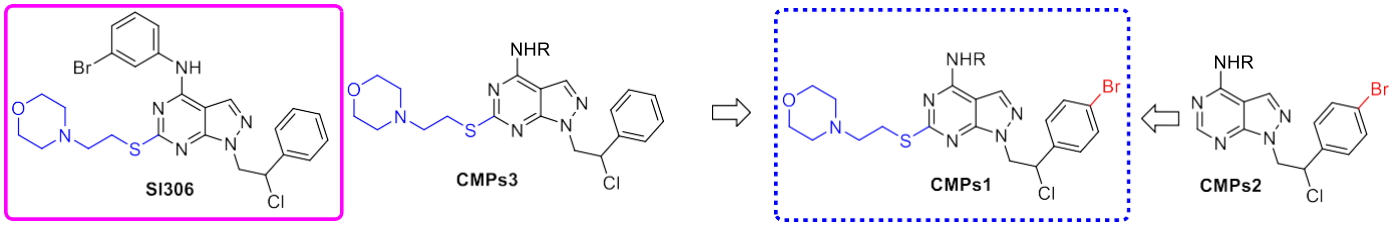

Figure 1. Structure of **SI306** and rational synthesis of new compounds **1**.

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POSTER 76

A MULTI-TECHNIQUE APPROACH FOR THE STUDY OF THE INTERACTION OF SMALL MOLECULES WITH G-QUADRUPLEX NUCLEIC ACIDS

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Guanine-rich sequences are known to fold into non-canonical structures named G-quadruplexes (G4s).[1] The structure of G4 is regulated by *Hoogsten* base pairing of four guanines stabilized by monovalent cations at the center of the quartet and by the stacking of two or more of these square planar arrangements.[2] They are mainly present in telomeres and in promotorial region of oncogenes and their stabilization through the binding with small molecules inhibits telomerase activity and therefore cell immortalization and transcriptional machinery, respectively.[3]

Our group developed a protocol based on a combination of advanced analytical techniques for studying the ability of small molecules to interact with nucleic acids *in vitro*.

Electrospray ionization-mass spectrometry (ESI-MS) is a useful tool for the analysis of target-ligand binding thanks to the "softness" of the ionization technique in which minimal fragmentation occurs and non-covalent interactions are not altered. Several different parameters can be calculated from MS spectra, such as binding affinity, selectivity among different sequences and complex stability in terms of $E_{COM}^{50\%}$ thanks to collision-induced dissociation experiments.[4] On the other hand, nuclear magnetic resonance (NMR) can be used also to monitor the kinetics of a reaction and characterize the product formation in solution conditions that closely resemble the physiological environment. First, our group uses this approach to study the interaction between the small molecules and the nucleic acid: in a simplified model, the reaction between the ligand and a nucleobase such as guanine can be observed.[5] Moreover, when the 3D structure of a nucleic acid is resolved for a specific sequence, 1D titrations with the compound can be performed in order to see which parts of the arrangement are involved in the binding and therefore to gain insights on the binding motif in real time.

With this contribution, we aim at highlighting that the combination of different techniques is fundamental for fully understanding the binding of small molecules to G4.

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POSTER 77

VENOMPRED 2.0: AN INNOVATIVE IN SILICO PLATFORM FOR MULTIPLE AND STRUCTURALLY INTERPRETABLE TOXICOLOGICAL EVALUATIONS OF SMALL MOLECULES

Di Stefano, M.;[a,b] Galati, S.;[a] Granchi, C.;[a] Mancini, M.;[c] Fratini, F.;[c] Poli, G.;[a] Macchia, M.;[a] Tuccinardi, T.[a]

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The application of artificial intelligence (AI) and machine learning (ML) methods is taking ground in the fields of *in silico* toxicology and drug design, representing a promising solution for evaluating the safety profile of compounds, especially in lead optimization and ADMET studies, and to comply with the principles of the 3Rs calling for the replacement, reduction and refinement of animal testing. In this context, we herein present the development of VenomPred 2.0,[1] our freely available web tool that represents a powerful web-based platform for multiple and structurally interpretable toxicity predictions. VenomPred 2.0 maintains the user-friendly features of VenomPred,[2] thus allowing its full accessibility even to non-expert users, and allows to evaluate the potential carcinogenic, mutagenic, hepatotoxic, estrogenic, androgenic, skin and eye irritating effect of small molecules, as well as their acute oral toxicity, for a wide *in silico* toxicological profiling. All predictions are preformed through a consensus of multiple ML models, carefully optimized and selected among thousands of models combinations, to achieve a high predictive reliability. Moreover, by employing a new utility based on the Shapley Additive exPlanations (SHAP) method, VenomPred 2.0 allows the user to identify the specific structural moieties that are associated with the predicted toxicological effects of a molecule, thus highlighting potential toxicophores. Users can simply load on our platform (http://www.mmvsl.it/wp/venompred2/) the SMILES strings of the desired compounds, which can also be obtained by drawing the corresponding molecular structures on the platform sketcher. This way, it is possible to easily and rapidly obtain information about the toxicological profiling of the desired compounds and about the possible structural fragments associated to their toxicity (Figure 1).

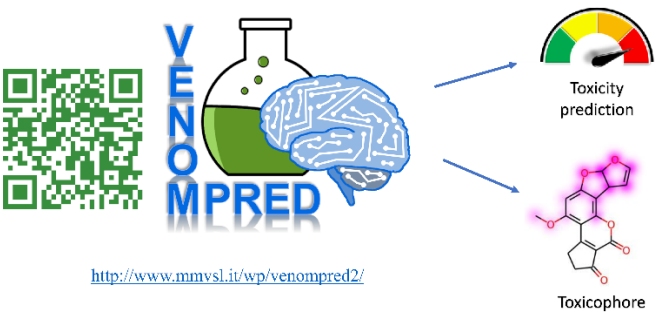

Figure 1. VenomPred 2.0 platform allows to evaluate both the toxicological profile and the possible toxicophores.

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POSTER 78

IDENTIFICATION OF NOVEL AMINOPYRIMIDINE DERIVATIVES AS PROTEIN KINASE INHIBITORS BLOCKING CELL GROWTH

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Aminopyrimidine scaffold is typical of some of the most promising anticancer drug recently discovered thanks to their ability to inhibit different types of protein kinases. Kinase deregulation has emerged as a relevant mechanism by which cancer cells evade normal physiological constraints and kinases inhibitors have become one of the most intensively pursued classes of recent antitumoral drugs.[1] Owing to the significance of pyrimidine derivatives as anticancer agents through kinase inhibition and our longstanding expertise in the development of pyrimidine derivatives, we designed and synthesized various classes of anilino and bis-anilinopyrimidines.[2] Most of them were found active in *in vitro* HTRF inhibition assays in low nanomolar range against one or more kinases, like EGFR, c-KIT, VEGFR, PDGFR, Akt and AURKA, wild type or mutated and double-mutated isoforms. Some compounds were also crystallized in the active site of some kinases, showing a preference for DFG-in or DFG-out conformation. Subsequently, the antitumor activity of selected compounds was evaluated on three different human cancer types chosen on the basis of their unsatisfactory therapeutic strategies and poor prognosis: glioblastoma multiforme, triple-negative breast cancer, colon adenocarcinoma, tongue squamous carcinoma and hypopharyngeal squamous carcinoma. Various pyrimidines demonstrated to also hinder cell proliferation and cell cycle and to induce apoptosis in all the tested cell lines, without exerting cytotoxic effects at the same concentrations. The data coming from the biological assays will be shown and discussed.

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POSTER 79

ARYLALKANOLAMINES AS SIGMA 1 RECEPTOR ANTAGONISTS USEFUL FOR THETREATMENT OF NEUROPATHIC PAIN

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Neuropathic pain (NP), a debilitating disorder caused by a damage or an impairment of the nerve pain signalling pathways, and particularly chemotherapy-induced peripheral neurotoxicity (CIPN), a pathologic condition that frequently occurs in cancer patients, are associated with severe, chronic pain for which no treatment is available sofar. Sigma 1 Receptors (S1Rs) have been strongly correlated to neuropathic pain, since their inactivation may decrease allodynia or dysesthesia, promoting an analgesic effect. S1Rs have a crucial role in chronic pain, in virtue of their ability in modulating the activities of genes or proteins (e.g. substance P receptor, NK1R), involved in NP. [1] In this study, we present the synthesis and biological evaluation of a series of S1R antagonists based on a 2-aryl-4- aminobutanol scaffold.[2] After assessing affinity towards S1R and selectivity over Sigma 2 Receptor (S2R), we investigated the compounds' agonist/antagonist profile by studying their effects on both nerve growth factor- induced neurite outgrowth and aquaporin-mediated water permeability in the presence and absence of oxidative stress. Among the compounds evaluated, *(R/S)*-**RC-752** emerged as the most promising candidate due to its high affinity for S1R and functional antagonist activity. Importantly, *(R/S)*-**RC-752** exhibited no cytotoxic effects in two normal human cell lines and a zebrafish model in vivo. Additionally, it remained stable after incubation in mouse plasma.

Based on these results, *(R/S)*-**RC-752** was moved further in preclinical studies and its efficacy in treating NP was weevaluated in two animal models: the formalin test and the spinal nerve ligation model. The results demonstrate that *(R/S)*-**RC-752** effectively alleviates pain in both animal models, thus providing proof-of-concept for its potentialas an antinociceptive agent. These findings highlight the therapeutic promise of *(R/S)*-**RC-752** as a novel non-opioidtreatment option for NP.

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POSTER 80

DISCOVERY OF QUINOLINONYL DERIVATIVES AS ANTI-HIV-1 INHIBITORS ENDOWED WITH AN INNOVATIVE MECHANISM OF ACTION

Madia, V. N.;[a] Ialongo, D.;[a] Messore, A.;[a] Patacchini, E.;[a] Arpacioglu, M.;[a] Scipione, L.;[a] Kvaratskhelia M.;[b] Di Santo, R.;[a] and Costi, R.[a]

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HIV integrase (IN) is a pivotal antiretroviral drug target. In this regard, IN strand transfer inhibitors (INSTIs), binding to the IN active site, have proven to be highly effective, becoming a potent first-line therapy to treat infected patients. However, despite their effectiveness as therapeutic options and the high barriers with the second-generation FDA-approved INSTIs, drug therapy selects for drug resistance and mutations responsible for multiple INSTIs resistance, underscoring the need for the development of more effective antiretroviral compounds. The development of small molecule protein-protein interaction inhibitors is a new attractive strategy for discovering anti-HIV agents. In this field of research, allosteric IN inhibitors (ALLINIs), are a promising new class of antiretroviral agents. These inhibitors act differently in respect to INSTIs, in fact, they alter the functional IN multimerization. Recently, it was unraveled that aberrant IN multimerization underlies the inhibition of IN-vRNA interactions by ALLINIs.[1] In doing so, ALLINIs indirectly disrupt the IN-vRNA binding, leading to the formation of defective viral particles with greatly reduced infective potential with mis-localization of the vRNA outside the viral capsid.[2] While the indirect disruption of IN-vRNA binding (caused by the impairment of functional IN multimerization) has been described with the treatment of virus-producing cells with ALLINIs, the direct disruption of this binding (without affecting IN multimerization properties) by small molecules has not been reported so far. We describe a series of compounds identified as inhibitors of the IN-vRNA binding via a direct mechanism. In particular, we deepened the mechanism of action of some compounds previously described by us as INSTIs.[3] Indeed, we speculated that these quinolinonyl derivatives, being endowed with two DKA chains, could also act as protein-nucleic acid interaction inhibitors. To verify our hypothesis, we decided to test a set of derivatives and their analogues endowed with a variable "base-like" functional group. We assessed the capability of our derivatives of inhibiting at low micromolar concentrations both IN 3'-processing and strand transfer reactions in a LEDGF/p75 independent assay. In addition, we performed in vitro binding assays, and we found that our quinolinonyl derivatives are able to disrupt the IN-vRNA interaction, that is vital for a correct generation of a functional infective virion. The data coming from the biological assays will be shown and discussed.

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POSTER 81

DISCOVERY OF NEW SMALL MOLECULES AS ANTI-SARS-CoV-2 AGENTS INHIBITING ACE2-SPIKE BINDING

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The severe and acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a plus-strand RNA virus closely related to SARS and MERS, rapidly spreading worldwide. This virus is responsible for an ongoing global pandemic of coronavirus disease 2019 (COVID-19) causing many casualties in the human population. For these reasons, it is imperative to find drugs able to solve this global health issue as fast as possible. Interaction between the spike (S) protein of SARS-CoV-2 and the cell surface receptor angiotensin-converting enzyme 2 (ACE2) is responsible of the infectivity of the host, allowing the entry of the virus into the host cells. Indeed, ACE2 acts as a ligand-receptor pair that initiates the viral attachment and cellular entry of the virus. In particular, the receptor-binding domain (RBD) of the S protein binds the membrane-distal portion of the ACE2 protein.[1] Notably, current vaccines induce antibody responses to S protein, and most neutralising antibodies bind to the RBD. Therefore, targeting the binding between the S protein and the ACE2 receptor is a promising approach for virus entry.[2] Inhibitors of the protein-protein interaction between the S protein and human ACE2 are of considerable interest as potential antiviral agents because the interaction between S and ACE2 initiate membrane fusion and virus entry, taking place at an accessible extracellular site.[3] While development of protein-protein interaction inhibitors with small molecules is more challenging than antibodies, small-molecule inhibitors could offer alternatives that are less strain- and mutation-sensitive, suitable for oral or inhaled administration, and more controllable/less immunogenic. Indeed, this strategy has already been successfully applied to inhibit the viral entry of other viruses, as in the case of two FDA-approved drugs maraviroc and enfuvirtide. This work was aimed at finding potent antiviral drugs against SARS-CoV-2 by targeting well identified proteins essential to the viral cycle, using HTS techniques. A flagged version of the recombinant RBD of the SARS-CoV-2 and ACE2 receptor with two different tags constructed by collaborators IP Paris was used for the HTS to identify molecules that interfere with the interaction between the RBD and ACE2. 21 candidates were selected from HTS based on a robust HTRF assay and, among them, two hits were identified, showing % of inhibition of 51.4 and 25.6 and IC_{50} of 1 and 2.42 µM *vs* ACE2 and of 0.44 and 3.58 µM against RBD. However, the two hits proved to be cytotoxic against SARS-CoV-2 infected cells. Therefore, in order to reduce the cytotoxic profile of these compounds and to improve their druggability, some analogues were designed. The data coming from the biological assays will be shown and discussed.

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POSTER 82

DEVELOPMENT OF NOVEL CARBONIC ANHYDRASE INHIBITORS AS ANTICANCER AGENTS FROM TUBULIN ASSEMBLY SCAFFOLDS

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes that catalyse the reversible conversion of carbon dioxide and water to bicarbonate and a proton via a coordinated metal ion. Hence, CAs activity is pivotal to the metabolism regulation under both physiological and pathological conditions.[1] The human CA (hCA) isoforms have been an ever-growing focus of attention as a therapeutic strategy for the design of new agents to treat a variety of diseases, including cancer.[2]

Herein, in an effort to identify novel carbonic anhydrase inhibitors as anticancer agents, we designed and synthesised a series of structurally related indole and pyrrole compounds by modulating the scaffold of the most active tubulin assembly inhibitors reported in our previous work.[3] Among the tested derivatives, we discovered that compounds **1** and **2**, characterised by the presence of both *N*1-(4-benzenesulfonamide) and 3-(3,4,5-trimethoxyphenyl) moieties, showed a strong hCA inhibition (Figure 1). These latter demonstrated to be equipotent to 5-FU as inhibitors of HCT116 cells, and to be remarkably more potent against the SW480 and SW620 cell lines. Noteworthy, compound **1** proved to be a novel dual-targeting drug, with activity against hCA and Wnt/β-catenin. Finally, molecular modeling studies highlighted docking poses, in the binding sites of the four hCA isoforms, consistent with the observed K_i values of this novel class of compounds.

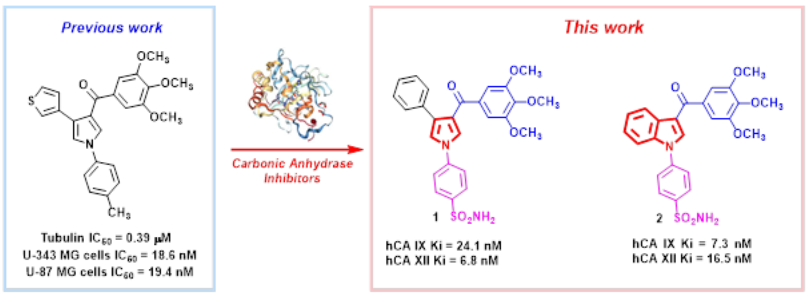

Figure 1. Rationale behind this work. Structure of compounds **1** and **2**.

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POSTER 83

LEAD OPTIMIZATION OF THE DIARYLALKYL AMINE SERIES AS DUAL REV-ERB AND AUTOPHAGY INHIBITORS WITH IN VIVO ANTICANCER EFFICACY

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We previously demonstrated that a dual inhibition of circadian nuclear receptor REV-ERBβ and autophagy represents a better anticancer approach than the single inhibition of autophagy.[1, 2] The first class of dual REV-ERBβ and autophagy antagonists is exemplified by our proprietary compounds **1a** and **1b** (**Figure 1**).[3, 4] Despite the enhanced potency and improved 'drug-likeness' of **1b** compared to the initial hit **1a**, its moderate metabolic stability limits its use in *in vivo* studies. Herein, we present our lead optimization strategies which involved the chemical exploration of the three main regions of **1c** (*regions A*, *B* and *C*, **Figure 1**). These studies led to the identification of an optimized compound with an improved biological profile, optimal 'drug-like' properties and efficacy in a mouse xenograft model of melanoma as a single anticancer agent.

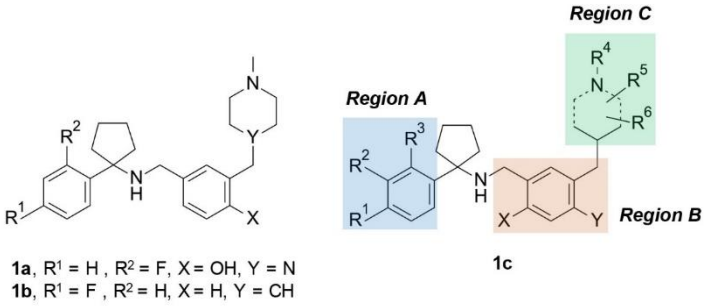

Figure 1

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POSTER 84

DESIGN AND SYNTHESIS OF TETRAHYDRO-β-CARBOLINES DERIVATIVES AS PDIA INHIBITORS

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Protein disulfide isomerases (PDIs) are multifunctional chaperones structurally related enzymes which catalyze disulfide bonds formation, reduction, or isomerization of newly synthesized proteins in the lumen of the endoplasmic reticulum (ER). In humans the PDI family, is composed of 21 members classified by sequence and structural homology.[1] The first identified member of this protein family is PDIA1 and it is structurally characterized by two thioredoxin-like active domains (a, a'), two substrate-binding domains (b, b') with a hydrophobic pocket in the b' domain, a linker sequence between the b' and the a' domains, and a C-terminal extended domain.[2] Structure related with PDIA1, ERp57/PDIA3, shows considerable overlap in their entire protein structure. However notable differences concern their different cellular biochemical roles in cellular homeostasis. ERp57/PDIA3 is involved, as the other PDIs, in the proper folding and in the formation and reshuffling of the disulfide bridges of the proteins synthesized in the rough ER. Considering these functions, it is not surprising that PDIA3 has been associated with several human diseases, such as cancer, prion disorders, neurodegenerative diseases, hepatitis, metabolic diseases, musculoskeletal system conditions, airway inflammation, platelet aggregation , viral infection , and PDIA3 expression level has been evaluated as a useful biomarker for diagnosis and/or prognosis in several conditions. Therefore, PDIA3 may be an interesting pharmacological target. Previously, **16F16** was reported as an irreversible inhibitor of PDIA1 and PDIA3 proteins due the presence of a chloroacetyl group that covalently modifies free cysteine thiols.[3] Starting from the chemical structure of **16F16**, we designed and synthesized a small set of structural analogues, characterized by a tetrahydro-β-carbolines core endowed with a chloroacetyl group in 2-position as PDIA3 inhibitors, to further explore the structure activity relationship (SAR). The data coming from the biochemical assays will be shown and discussed.

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POSTER 85

COMPUTER-AIDED IDENTIFICATION, SYNTHESIS, AND BIOLOGICAL EVALUATION OF DNA POLYMERASE η INHIBITORSFOR THE TREATMENT OF CANCER

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Chemoresistance is one of the main reasons why a definitive therapy for cancer has not yet been identified. Chemotherapeutic drugs can in many ways disrupt the replication machinery triggering apoptosis in cancer cells: some act directly on DNA and others block the enzymes involved in preparing DNA for replication. Cisplatin-based drugs are common as first-line cancer chemotherapics. These drugs act by forming a covalent interaction with DNAleading to cancer cell death. Another example is etoposide, a molecule that blocks topoisomerase II α leading to theinhibition of dsDNA replication. Despite their efficacy, cancer cells can respond to these treatments over time by overtaking their effects, leading to drug resistance. This phenomenon is referred to as chemoresistance. Chemoresistance events can be generated by the action of TransLesion DNA Synthesis (TLS) enzymes, like DNA polymerase η (Pol η). Pol η belongs to the Y family of polymerases, this class of Pols allows DNA duplication even indamaged regions along the nucleic acid strands. In particular, Pol η is exceptional in its ability to bypass specific DNA damages like cyclobutane pyrimidine dimers (CPDs), which can be caused by UV light. This polymerase helps also tobypass drug-induced damage in cancer cells, allowing DNA replication and cancer cells proliferation even when cisplatin-based chemotherapeutic drugs are in use. For these reasons, Pol η is a promising target, whose inhibition would help in the overcoming of drug resistance. This study was aimed at the discovery of a potent and selective Pol η inhibitor to improve the efficacy of platinum-based chemotherapic drugs. So far, we found a novel small- molecule. Compound 64 (ARN24964), derived from a natural compound the luteolin, is an inhibitor of Pol η able toact synergistically with cisplatin. We also evaluated its activity on three different cancer cell lines, for these reasons,this compound represents an advanced scaffold featuring good potency, aqueous solubility, microsomal and plasmastability.

POSTER 86

IPERIDINO-BASED BENZENSULFONAMIDE DERIVATIVES AS CARBONIC ANYDRASE INHIBITORS

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Selective targeting of the tumor associated human carbonic anhydrase CA IX and CA XII isoforms become extremely important for the development of new potential antiproliferative compounds for the treatment of solid tumors.[1] New chemical entities with a significant degree of isoform selectivity is required for the treatment of target diseases reducing side effect related to promiscuous CAs inhibition. In this context the ureidobenzenesulfonamide SLC-0111, a selective CA IX inhibitor, is currently in Phase Ib/II clinical trials in combination with Gemcitabine in subjects affected by metastatic pancreatic ductal adenocarcinoma, overexpressing CA IX/XII.[2] Starting from SLC-0111 scaffold we incorporated the ureido moiety into the piperidine heterocyclic ring system. Furthermore, we studied the impact of different groups as tail of the inhibitor, combining hydrazidoureido, hydrazidothioureido, hydrazone and amide moieties (Figure 1.).[3-5]. All the molecules were further tested against the cytosolic hCA I and II isoform, as well as the transmembrane, tumor-associated enzymes hCA IX and XII, showing broad spectrum of activity and selectivity which is strictly dependent with the substitution in the tail region.

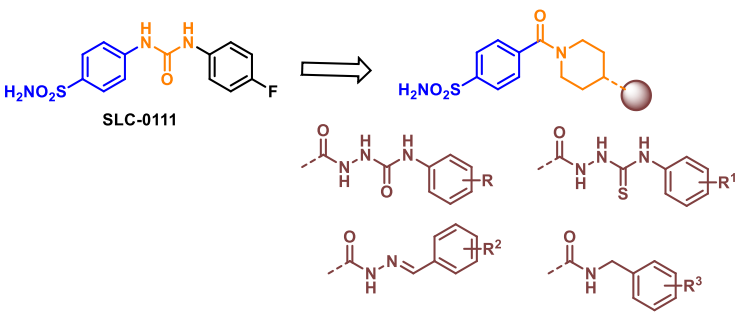

Figure 1. Piperidinobenzenesulfonamide derivatives design

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POSTER 87

MULTIPOTENT CHROMONE-BASED AGENTS INTERFERING WITH ARACHIDONATE PATHWAY

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Acute inflammation is a natural response of the body to restore homeostasis after different external stimuli. However, a long-term exposition can turn it in a chronic inflammation, which plays a fundamental role in various chronic diseases, including several neurodegenerative diseases characterized by neuroinflammation. The arachidonic acid (AA) signaling pathway, involving multiple enzymes and metabolites, is a trailblazer in the pathogenesis of inflammation. COXs and LOX are crucial AA metabolizing enzymes: COX-2 levels are increased at the site of inflammation, especially in early stages of the diseases; 15-LOX is responsible for the biosynthesis of eoxins, involved in several inflammatory conditions. The simultaneous and selective targeting of COX-2 and 15-LOX could thus be a promising strategy to counteract (neuro)inflammation, circumventing the gastrointestinal side effects associated with the inhibition of COX-
1. The aim of the work is to combine different pharmacophores that proved to efficiently interact with the two enzymes. The chromone scaffold (a, fig. 1) is a natural-derived privileged structure with a wide range of activities, including anti-inflammatory and neuroprotective;[1] appropriate substitution with a methoxy group was considered, inagreement with compounds found in literature. The *N*-acylhydrazone fragment is a key pharmacophore for COX inhibition, and aiming at modulating its reactivity, it was fused with substituted carbamate moieties to originate carbamate-hydrazone fragments (b, fig. 1). These two synthones were merged to obtain a series of hybrids, which showed interesting potencies and selectivity profiles (fig. 1). A docking study was also performed for the most promising compounds on COX-2 and 15-LOX enzymes to elucidate their binding mode and further studies are in progress to evaluate their in vivo anti-inflammatory profile.

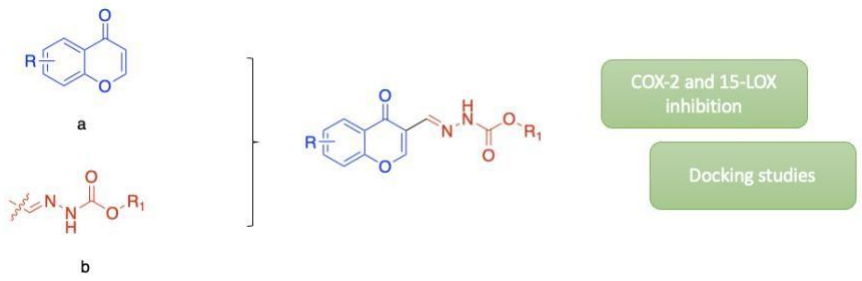

Figure 1. Design of hybrid antiinflammatorycompounds

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POSTER 88

NEW *N*-(HETEROCYCLYLPHENYL)BENZENESULFONAMIDES AS β-CATENIN INHIBITORS

Parisi, A.;[a] Nalli, M.;[a] Coluccia, A.;[a] Liu, T.;[b] La Regina, G.;[a] Silvestri, R.[a]

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Despite intensive efforts, no inhibitors of the Wnt/β-catenin signaling pathway have been approved so far for the clinical treatment of cancer. We synthesized novel *N*-(heterocyclylphenyl)benzenesulfonamides as β-catenin inhibitors (Figure 1).[1]
In crystallographic studies of the β-catenin armadillo repeats domain, compound **1** superimposed to Tcf-4 (PDB ID 2GL7) highlighting a common binding site within the hotspot binding region close to Lys508. To our knowledge, compound **1** is the first small molecule ligand of this region to be reported. In co-immunoprecipitation study in cells transfected with Myc-tagged Tcf-4, compound **1** abrogated the association between β-catenin and Tcf-4. Compound **1** induced *in vitro* cell death in SW480 and HCT116 cells and *in vivo* tumorigenicity of a human colorectal cancer line HCT-116.
These results highlight the potential of this novel class of β-catenin inhibitors as anticancer agents.

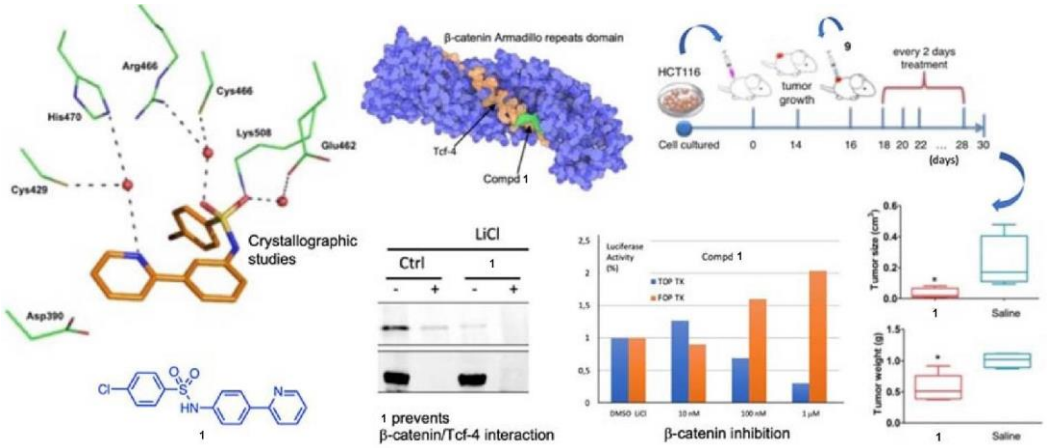

Figure 1. Structure and Biological Activity of Compound **1**.

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POSTER 89

A MIXED LIGAND-BASED AND STRUCTURE BASED PROTOCOL TO SELECT NEW POTENTIAL ANTI-COVID COMPOUNDS

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Since the early 2000's Coronaviruses have periodically been jeopardizing public global health and the main protease (M^{pro}) has been gaining more and more attention for its high degree of conservation among all family members and its essential role for their replication cycle. In addition, the peculiarity of its specific cleavage site promotes it as an attractive target to develop highly selective and tolerable broad-spectrum inhibitors.[1] In order to reduce costs and resources in the drug discovery process and speed up the prioritization of compounds selection for further synthesis and biological evaluation, the present project aimed to discover and rationally design new M^{pro} inhibitors through a combined usage of quantitative structure-activity relationship (QSAR)-based virtual screening (VS) and ligand-based (LB) and structure-based (SB) 3-D QSAR techniques. Therefore, relevant available data from current literature and databases in the form of chemical descriptors and molecular fingerprints were used to build QSAR models using several machine learning algorithms. The models were extensively validated and the best ones were applied in a sort of consensus protocol to VS the Enamine commercial database[2] of approximately 1 million of compounds. As ultimate filters were used Comparative Molecular Field Analysis like (CoMFA-like) and Comparative Binding Energy analysis like (COMBINE-like) models generated and validated through the *www.3d-qsar.com* web server.[3] The models endowed with the most relevant statistical results ($r^2 > 0.9$, $q^2 > 0.8$) were used to screen the VS selected compounds and identifying some promising potential leads. Furthermore, the graphic feature of the 3-D QSAR techniques led to depict some comprehensive SAR rules to design novel broad-spectrum inhibitors and drive a future hit-to-lead optimization. Further details will be presented during the poster session.

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POSTER 90

TARGETED METABOLIC PROFILING OF BRAIN-DERIVED CELL CULTURES BY SEMI-AUTOMATED MICROEXTRACTION BY PACKED SORBENT (MEPS) AND LC-MS/MS

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Endogenous metabolites involved in different pathways could play crucial roles in key physiological functions, and their imbalances may be implicated in central nervous system pathologies and neurodegenerative diseases. The feasible and reliable measurement of a wide array of metabolites and biomarkers possesses great potential to elucidate physiological and pathological mechanisms, aid preclinical drug development and highlight potential therapeutic targets.[1] An effective, straightforward, sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach was developed for the simultaneous quali-quantitative analysis of 41 compounds in both pellet cells and cell growth medium obtained from brain-derived cell cultures. Sample pretreatment miniaturisation was achieved thanks to the development and optimisation of an original extraction/purification approach based on digitally programmed microextraction by packed sorbent (eVol®-MEPS). MEPS allows satisfactory and reproducible clean-up and preconcentration of both low-volume homogenate cell pellet lysate and cell growth medium with advantages including, but not limited to, minimal sampling handling and method sustainability in terms of sample, solvents, and energy consumption. The MEPS-LC-MS/MS method showed good sensitivity, selectivity, linearity, and precision. The developed method was then successfully applied to the analysis of both cell pellet cell growth medium obtained from Oli-neu oligodendroglial precursor cell line cultures, leading to the unambiguous determination of all the considered target analytes. Within this multidisciplinary project, the developed methodology will allow the comparative assessment of biochemical processes involved in mitochondrial aspartate/glutamate carrier isoform 1 (AGC1) deficiency, a rare genetic disease leading to a severe encephalopathy in early childhood.[2] This will in turn clarify altered biochemical pathways, thus potentially highlighting the presence of specific biomarkers that could play a role in the onset and progression of the disease and address promising therapeutic targets.

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POSTER 91

DB.3D-QSAR.COM. THE FIRST 3D QSAR MODELS DATABASE

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3D QSAR is a computational approach used in drug design and molecular modeling to analyze the relationship between the three-dimensional structure of a molecule and its biological activity. It aims to understand how different structural features of a molecule contribute to its activity or potency. The process of 3D QSAR involves several steps. First, a dataset of structurally diverse molecules with known biological activities (BAs) is selected. Then, their three-dimensional structures are generated using computational methods. Next, in the classical form of Cramer[1], sterical and electrostatic molecular interaction fields (MIFs) are calculated and as final step a mathematical model is built through the correlation of BAs with MIFs by means of projection of latent structures (PLS) algorithm. With our interest of making 3D QSAR accessible to all as with the www.3d-qsar.com site[2], here is presented db.3d-qsar.com the first publicly available database of 3D QSAR models in which the user can insert or draw a molecule and predict its potency against an available target. All the models available in db.3d-qsar.com have been heavily optimized in prediction power through a semi-systematic pretreatment and parameter selection procedure by initially dividing the datasets in training (80%) and prediction (20%) sets. Each model to be opened to the public was selected among thousands of trials. The selected models were finally characterized using a validation set compiled with molecules taken from the ChEMBL database. At the time of writing more than 40 models associated to more than 30 different pharmacological targets have been prepared and ready to be used. At the time of the presentation the db.3d-qsar.com will be accessible to the public and during the presentation its features will be shown.

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POSTER 92

TARGETING IDO1 MOONLIGHTING ACTIVITIES BY DIFFERENT MODALITIES IN CANCER IMMUNOTHERAPY

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Background. **Indoleamine 2,3-dioxygenase 1** (**IDO1**), an attractive target for **cancer immunotherapy**, is a **moonlighting** protein that, besides being a tryptophan-metabolizing enzyme, behaves as a complex signal transducing molecule and exists in dynamic equilibrium between two forms: a heme-bound form (holo) and a heme-free one (apo)[1]. *Aims and results*. i) ***Development of drug-like apo-IDO1 inhibitors:*** VS-13 is a potent apo-IDO1 inhibitor (A375, IC_{50} = 16 nM) that was identified in our laboratory by a virtual screening approach, but suffers from **poor metabolic stability** in mouse liver microsomes (MLM, 32% residual substrate after 1 h of incubation)[2]. Hence, a SAR/SPR exploration around VS-13 was carried out to increase its ADME profile. A significant increase of metabolic stability was reached by combining deuterium incorporation[3] and isosterism. ii) ***Synthesis of PROTACs[4]:*** a sustainable and environmentally friendly platform based on multi-component reactions was exploited to synthetize in one single step a library of PROTACs characterized by linkers and linkage points of different nature and length[5]. Enzymatic assays and Western Blot analysis are on-going on the synthesized protein degraders. iii) ***Development of small molecules that modulate both enzymatic and signalling activities:*** VS-15 (A375, IC_{50} = 480 nM) represents the first apo-inhibitor that negatively modulates the SHP-mediated signalling activity of IDO1, with a great potential for decreasing IDO1 *de novo* synthesis and expression. Unfortunately, the *in vivo* use of VS-15 is hampered by low metabolic stability (5% residual substrate after 1 h in MLM). Therefore, a medicinal chemistry campaign has been undertaken and has led to the discovery of a drug-like lead compound that is being preclinically evaluated.

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POSTER 93

SCREEENING OF NATURE-INSPIRED PHOSPHODIESTERASE 9 INHIBITORS TARGETING THE CENTRAL NERVOUS SYSTEM

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As life expectancy increases, the need for novel tools against cognitive decline emerges. Worldwide, 44 million people are affected by dementia, and this number is predicted to triple by 2050. Currently used small molecules target acetylcholinesterase and N-methyl-D-aspartate receptors, but limited efficacy is reported against Alzheimer's disease.[1] In the past decades, neuroprotection and cognitive enhancement have been related to the potentiation of cAMP and cGMP signaling in the brain. These events can be promoted by the inhibition of phosphodiesterases (PDEs), and ultimately result in neurotransmitter release, amelioration of microvascular dysfunction and neuronal plasticity. In particular, cGMP-selective enzymes are attracting growing attention as potential targets to modulate signal transduction.[2,3]
In this contribution, the discovery of PDE9 inhibitors is described, based on the features of this isoform with high affinity for cGMP that encourage the development of neuroprotective agents: its expression in the brain, the presence within its structure of a peculiar accessory binding pocket, the asymmetry between the two subunits composing the protein dimer, and the selectivity towards chiral species. In this context, the world of natural and Nature-inspired compounds represents a source of chemically divers scaffolds.[4] In our study, ligand- and structure-based *in silico* techniques have been applied for the identification of drug-like, CNS-targeting PDE9 inhibitors from natural sources.[5] Namely, prediction of physico-chemical descriptors, docking and molecular dynamics studies were enrolled. Moreover, *in vitro* PDE9 inhibition assays were performed to parallel the computational results.

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POSTER 94

STRUCTURAL MODIFICATIONS OF NOVEL POTENT AGONISTS OF GPR55 RECEPTOR BEARING A 3-BENZYLQUINOLIN-2(1*H*)-ONE STRUCTURE

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GPR55 is a G protein-coupled receptor (GPCR) that is widely distributed throughout human tissues, with high expression levels in the central nervous system (CNS), including neurons, astrocytes, and microglia. Although its classification and exact function in the CNS is still under investigation, several studies have shown that dysregulation of GPR55 expression and activation can contribute to the pathogenesis of neurodegenerative diseases by promoting neuroinflammation, a hallmark of many CNS disorders. As a result, research has recently focused on developing novel and potent GPR55 modulators as potential innovative therapeutic agents for neurodegenerative diseases.[1]

Recent studies carried out in our lab have focused on the synthesis of a first series of variously substituted 3-benzylquinolin-2(1*H*)-ones as potent GPR55 agonists (*figure 1*, **A**).[2] The elongation/shortening or ramification of the 7-*n*-butyl sidechain or the removal of the R_{11} benzyl substituent (*figure 1*, **B**) led to a second series showing, in some cases, a functional shift towards inverse agonism.

In this work we present the selectivity data of the compounds of the second series (**B**) for GPR55 over both cannabinoid receptors CB1 and CB2 and we synthesize a further series of compounds in which the methoxy substituent of the benzyl group is shifted from the *ortho* (R_{11}) to the *meta* position (R_{12}) (*figure 1*, **C**).

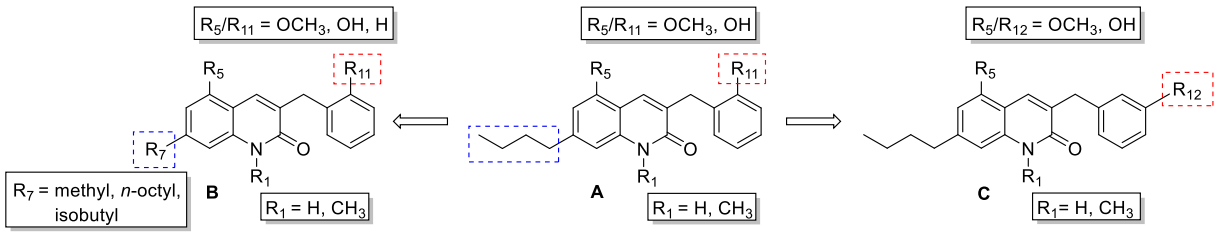

Figure 1. Structural modifications applied to the novel class of 3-benzylquinolin-2(1*H*)-ones.

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POSTER 95

TET2 AS A PROMISING TARGET FOR THE TREATMENT OF AGGRESSIVE MASTOCYTOSIS

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TET2 dioxygenase is an epigenetic factor able to oxidize methyl-cytosines, the first step towards DNA demethylation. Its mutations are prevalent in a variety of human haematological malignancies.[1] Among these, it has been shown that TET2 loss-of-function cooperates with the activation of the oncogenic cKIT (KITD816V) to drive aggressive mastocytosis, a rare disease characterized by the abnormal accumulation and activation of mast cells with damaging effects on the organs and tissues in which they reside. The molecular mechanisms underlying this functional cooperation are still unclear.[2]

Here, we show that TET2 wild-type is upregulated in the presence of KITD816V and transiently induced in response to a panel of different immune stimuli in mast cells. Moreover, genome-wide studies focused on transcription, methylation, histone modification, and transcription factor binding highlight that TET2 targets and regulates a subset of immune genes in mast cells activated by KITD816V mutation. In this context, TET2 deficiency leads to the repression of these immune genes resulting in immune tolerance to acute stimuli and enhanced survival of chronically activated mast cells. Finally, we tested the effect of vitamin C, an enhancer of TET2 processivity, on activated mast cells presenting TET2 mutation on a single allele. In this model, vitamin C is able to revert the mast cell phenotype boosting the residual activity of TET2.

Overall, our data support a model where TET2 plays a direct role in preventing immune tolerance in chronically activated mast cells and indicate TET2 as a promising target for novel treatments of aggressive mastocytosis.

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POSTER 96

NEW TEIXOBACTIN ANALOGUES CONTAINING LACTAM RING

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Teixobactin is a new antibiotic peptide isolated by the bacterium *E. terreae,* which shows high activity against Gram+ resistant bacteria, like *S. Aureus*, *MRSA*, *C. Difficile* and *Mycobacterium tuberculosis*.[1,2] These new molecules represent a new hope against the growing phenomenon of antibiotic resistance, but its complex structure and low water solubility represent a real challenge for industrial scale up and pharmaceutical formulations. Thus, researchers have focused their attention to the development of new efficient and simple synthesis of teixobactin analogues with better chemical and physical properties.[3,4] The aim of this study is the synthesis of new efficient teixobactin analogues containing a total lactam ring, by replacing D-Threonine[8] with (2R,3S)-diamino propionic acid (D-Dap), as showed in **Figure 1**. Products were successively tested on *S. Aureus*, *E. coli, MRSA* and *C. glabrata* with MIC test. Third, fourth and seventh analogues, all containing Nle[11], have shown a moderated interesting activity against *S. Aureus* and *MRSA.* MIC results are summarised in **Table 1**.

	***S. Aureus* ATC 25923**	***MRSA* ATC 33511**
D-Dap$_8$-Arg$_{10}$-Nle$_{11}$-Teixobactin	4	4
D-Dap$_8$-Arg$_{10}$-Nle$_{11}$-Teixobactin-H	4	4
D-Arg$_4$-D-Dap$_8$-Arg$_{10}$-Nle$_{11}$-Teixobactin	4	4

Table 1. MIC values of tested analogues. Results are expressed as μg/ml [b].

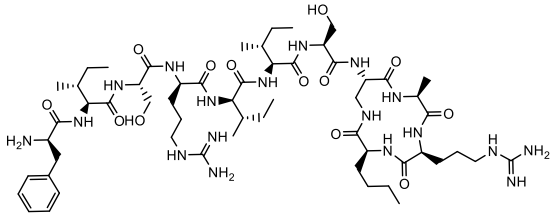

Figure 1. Lactam Teixobactin analogue.

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POSTER 97

IDENTIFICATION AND OPTIMIZATION OF NEW SARS-COV-2 MAIN PROTEASE INHIBITORS BY ANINTEGRATED IN-SILICO AND IN-VITRO STRATEGY

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The recent pandemic has underscored the continual threat of newly emerging and re-emerging pathogens andthe critical value of research in pandemic preparedness efforts. The accrual of solid knowledge and new therapeutics isurgently needed to enable a rapid response to future public health emergencies.

Due to its essential role in the viral life cycle and high conservation among different coronaviruses, we focused on the main protease of SARS-CoV-2, M^{pro}, as an appealing antiviral target.[1] We performed a structure-based virtual screening on a library of commercially available compounds, followed by the FRET-based biochemical screening of the best resulting chemical entities, in parallel with biophysical and crystallographic analyses on the isolated recombinant target.[2] We identified three highly promising M^{pro} inhibitors hits, characterized by good *in vitro* activity in the micromolar range and, above all, by innovative chemical scaffolds compared to the small molecules currently under development We thoroughly characterized our novel hits, elucidating i) the inhibitors-target recognition by state-of-art analytical and biophysical tools including X-ray crystallographic and native mass spectrometry studies, and ii) the antiviral profile confirming their antiviral activity in SARS-CoV-2 infected cells without causing cytotoxicity. Finally, an intensive campaign of chemical synthesis is ongoing to obtain a collection of optimized compounds structurally related to the identified hits.

Our results represent an improvement of the knowledge of the structure-activity relationships for M^{pro} inhibition while building preparedness valuable to face future medical threats from epidemic- or pandemic-prone emerging viruses.

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POSTER 98

NEW MULTIDRUG RESISTANCE REVERSERS ACTING AS BIOFILM INHIBITORS

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Biofilms are microbial communities encased in self-produced matrix composed of exopolysaccharides, proteins and DNA.[1] Nowadays, biofilms are recognized as an important issue in human disease management due to their resistance, achieving 10- to 1000-fold higher tolerance to antimicrobial agents than corresponding planktonic bacteria.[2] Biofilm resistance has a multifactorial nature resulting from the combination of several mechanisms, including restricted penetration of antimicrobials through the exopolysaccharide matrix, presence of Quorum Sensing (QS) factors and expression of genes encoding efflux pumps involved in resistance.[3] Among the different mechanisms involved in biofilm formation, we focused our project on Quorum Sensing (QS) factors and efflux pumps. Our aim was the design of new molecules directed against these mechanisms (Figure 1) and the evaluation of their ability to inhibit biofilm formation and to disrupt already formed biofilm in models of different strains belonging to different Gram-negative species.

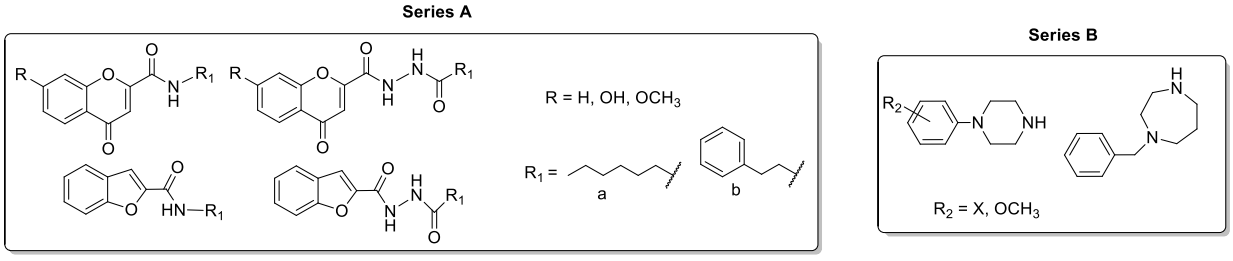

Figure 1. New molecules acting as biofilm inhibitors.

Compounds belonging to the A series were designed based on the structure of V-06-018, a potent antagonist of the LasR receptor involved in the QS mechanism; B-series derivatives were already described by us as efflux pump inhibitors in planktonic cells.[4,5] The ability of each molecule to inhibit biofilm formation has been evaluated on microtiter 96-well plates using crystal violet to stain adherent cells. Different strains of *Burkholderia cenocepacia* and *Escherichia coli* species have been used in the test. Preliminary results indicated the presence of a certain inhibitory activity on biofilm formation. Most promising derivatives will be tested also on *Pseudomonas aeruginosa*, which is the most prevalent pathogen of Cystic Fibrosis (CF) lung disease showing the biofilm formation as important adaptive mechanism.

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POSTER 99

TOWARD THE DEVELOPMENT OF PROTEOLYSIS-TARGETING CHIMERAS (PROTACs) DIRECTED TO PRION PROTEIN

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Prion diseases are fatal neurodegenerative disorders with no effective cure. The main pathological hallmark has been identified as conformational changes of the cellular prion protein (PrP^C) into misfolded isoforms (PrP^{Sc}). Targeting PrP^C and its conversion to PrP^{Sc} led to the identification of many small molecules with therapeutic potential.[1] Nonetheless, the search for effective anti-prion candidates remains highly challenging, due to strain heterogeneity and drug-resistance mechanisms. This scenario advocates pursuing new directions in prion drug discovery by harnessing novel therapeutic modalities. Hence, we sought to develop PrP-directed proteolysis- targeting chimeras (PROTACs), i.e., heterobifunctional small molecules able to induce PrP degradation, featuring a PrP ligand connected via a linker to an E3 ligase binder. As a PrP ligand, a derivative of diphenylmethane derivative GN8, which stabilizes PrP^C conformation and decreases PrP^{Sc} levels,[2] was selected. Thanks to the reported 3D structural information, we identified the suitable exit vector for linker derivatization, and E3 ligase ligand conjugation (Figure 1). The resulting PROTAC has been preliminarily evaluated in noninfected neuronal N2a cells and RML strain scrapie-infected N2a (ScN2a-RML) cells to assess cytotoxicity and anti-prion activity. While many questions and challenges remain, PROTAC-mediated PrP degradation may be an innovative and effective strategy to tackle prion diseases.

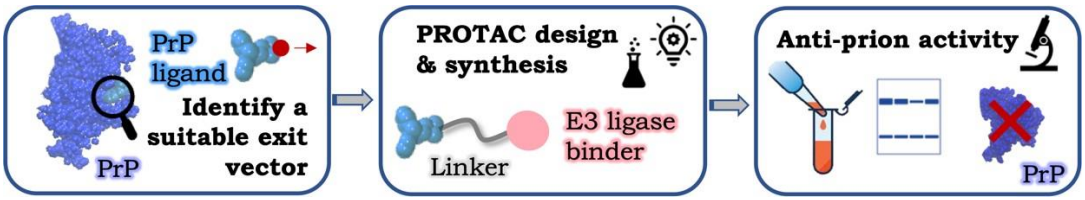

Figure 1. Development of PrP-directed PROTACs.

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POSTER 100

IDENTIFICATION OF BINDERS TO PRE-MIR-21 BY ^{19}F-NMR FRAGMENT-BASED LEAD DISCOVERY

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MicroRNAs (miRNAs) are a class of non-coding single stranded RNAs of 19-27 nucleotides (nt). They are synthesized starting from the DNA in a long primary miRNA sequence (pri-miRNA), which is then processed by the nuclear ribonuclease Drosha, into 60–70 nt stem-loop-structured precursor miRNAs (pre-miRNAs). These pre-miRNAs are transported into the cytosol where are processed by the Dicer enzyme into the mature miRNAs[1]. These non-coding RNAs are involved in the regulation of many cell/tissue processes and their deregulation is reported in more than 160 diseases[2,3]. In particular, miRNA-21 (miR-21) has an anti-apoptotic role and is upregulated in many tumours[4-6]. Therefore, its down regulation represents a potential therapeutic approach for the treatment of many tumors[7]. Indeed, small molecule modulators of miR-21 levels have been described in the literature[8]. Among the different approaches proposed for downregulating miR-21, inhibition of its maturation by targeting pre-miR-21, instead of Dicer, has emerged as a promising methods for obtaining selective compounds and not affect other miRNAs[9]. To find new chemotypes capable of decreasing the levels of miR-21 in cells, we applied a ^{19}F-NMR Fragment-based Lead Discovery approach[10,11] against pre-miR-21. We screened a small library (LEF, Local Environment of Fluorine)[12] of fluorinated fragments in mixtures of 20-25 compounds each, in absence and presence of pre-miR-21: compounds that bind to the target show a line broadening of their ^{19}F NMR signal and are easily identified[13]. The hits identified through NMR were further validated by Fluorescent Polarization binding assays. The compounds able to bind the pre-miR-21 using both approaches were then tested for their ability to inhibit Dicer cleavage in vitro. This workflow enabled the identification of small molecules as starting point for novel miR-21 modulators, which could be further developed for use in cancer therapy.

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POSTER 101

TARGETING GLUCOSYLCERAMIDE SYNTHASE TO FIGHT RESISTANCE TO OSIMERTINIB IN NON-SMALL CELL LUNG CANCER

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Simertinib (OSI) is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor which is selective for activating mutations in patients with advanced non-small cell lung cancer (NSCLC). Despite the strong clinical efficacy exerted by OSI, patients inevitably develop secondary resistances to this treatment.[1] In addition to the onset of genetic mutations, onco-metabolism has been proved to be involved in drug resistance development.[2] In the current study we evaluated the alteration of lipid metabolism associated with the onset of OSI-resistance in NSCLC cell lines. We performed a lipidomic analysis of OSI-resistant and OSI-sensitive NSCLC cell lines using Ultra High-Performance Liquid Chromatography coupled with Ion Mobility Separation and Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-IMS-QTOF MS). Multivariate analysis revealed a clear separation between OSI-resistant and OSI-sensitive cell lines and among the most discriminant variables we found glycosylceramides, which were significantly increased in OSI-resistant cells compared to the OSI-sensitive ones. Driven by these results we treated resistant cells with the glucosylceramide synthase (GCS) inhibitor, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) to assess its ability to overcome OSI-resistance. Targeting GCS using PDMP significantly reduced the levels of glycosylated ceramides and, at the same time, inhibited cell proliferation, impaired colony formation and migration and increased apoptosis in resistant cells. These results suggest that up-regulation of ceramide (poly-)glycosylation by GCS is involved in the maintenance of OSI-resistance. Moreover, the use of a GCS inhibitor may represent a promising strategy to treat EGFR-mutant NSCLC patients progressed to OSI-resistance.

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POSTER 102

PK7: A SYNAPTIC TARGETING COMPOUND TO OVERCOME PARKINSON'S DISEASE

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Parkinson's disease (PD), one of the most common neurodegenerative disorder, is nowadays an unmet clinical need, since the gold standard therapy, Levodopa, is efficacious in treating only the motor symptoms but shows adverse effects and lose efficacy with PD progression. We recently identified the neuronal phosphoprotein Synapsin III (Syn III), physiologically cooperating with functional alpha-synuclein (aSyn) to stimulate dopamine release[2], as a key component of aSyn pathologic aggregates[1], supporting that Syn III is pivotal for PD progression and that the pathological aSyn/Syn III interaction could constitute a therapeutic target for PD. Recent literature data suggest how the monoamine reuptake inhibitor methylphenidate (MPH) is able to permit the motor activity recovery in PD tg mice. We demonstrated how this promising activity is related to the re-establishment of the functional interaction between Syn III and α-helical aSyn[3]. Starting from MPH, we developed two generations of derivatives as disease-modifying agents[4a], which were *in vitro* screened using FRET, thus selecting PK7 as our lead compound. PK7, which drug-likeness was predicted, confirmed its ability to positively modulate the aSyn/Syn III complex, did not showed any cytotoxic effect and lost the off-targets effect on MATs, thus avoiding any side effect, common of MPH derived molecules[4b]. Our candidate was able to reduce aSyn aggregates, both *in vitro* and *in vivo*, showed the ability to exert neuroprotection and to restore motor ability[4b]. PK7 efficacy was evaluated also in midbrain organoids from PD patients and the *in vivo* PK/ADME properties were determined[4b], suggesting its strong potentiality for a Phase I clinical development.

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POSTER 103

HAZELNUT AND ITS BY-PRODUCTS: IN SILICO TARGET FISHING

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Hazelnut and its by-products have been extensively studied for their rich content of phenolic compounds, flavonoids, tannins, polysaccharides, and dietary fibers which possess strong antioxidant properties and exhibit various bioactivity potentials. Indeed, several studies have demonstrated the remarkable benefits of hazelnut consumption on cardiometabolic risk factors, surpassing expectations. Other research has shown that hazelnut consumption can reduce the risk of coronary heart disease, atherosclerosis, and certain cancers.[1] The identification of targets whose interaction is likely to explain the involvement of hazelnut components in these diseases is the goal of our work. In the current study we performed an exemplary application of a virtual parallel screening approach to identify potential targets for 45 secondary metabolites from the kernel and skin parts of the hazelnut. The applied parallel screening paradigm with constituents of hazelnut on different proteins has shown promise as an in silico tool for rational target fishing and pharmacological profiling of extracts and single chemical entities in natural product research. In vitro experiments will be performed in order to test the predicted theoretical activities. Our analysis could represent a good strategy to best take advantage of hazelnut by-products for the concerned industries, such as food, cosmetics and pharmaceutics; and, by consequence, for the scientific community.

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POSTER 104

IN SILICO-GUIDED RATIONAL DRUG DESIGN AND SYNTHESIS OF NOVEL 4-(THIOPHEN-2-YL)BUTANAMIDES AS POTENT AND SELECTIVE TRPV1 AGONIST

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Vanilloid receptor type 1 (TRPV1) is a ligand-gated non selective cation channel that plays a pivotal role in pain processing and neurogenic inflammatory response. Starting from the most promising compound identified in a previous work,[1] we described the in silico-guided rational drug design and synthesis of a series of 4-(thiophen-2-yl)butanamides as novel TRPV1 agonists. Most of the synthesized compounds showed high TRPV1 efficacy and potency as well as selectivity. The molecular modeling analysis highlighted crucial hydrophobic interactions between Leu547 and the iodo-thiophene nucleus, or between Phe543 and the pyridinyl moiety. In the biological evaluation, two compounds showed protective properties against oxidative stress-induced ROS formation in human keratinocytes. Additionally, one showed neuroprotective effects in both neurons and rat brain slices, while the other exhibited potent antinociceptive effect in vivo.[2] These results provide us with additional insights on the SARs for this novel class of 4-(5-substituted-thien-2-yl)-butanoic acid amides, paving the way for further chemistry optimization.

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