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Paestum (SA), 10-14 settembre 2017

➢ Divisione di Chimica Farmaceutica

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Programma Scientifico

Divisione di Chimica Farmaceutica

Lunedì 11 Settembre 2017

	Sala Saturno
Chairpersons: Gabriele Costantino, Fabrizio Giordanetto	
9:00-9:30	FAR KN01 - Fabrizio Giordanetto, D E Shaw Research *Fragment-based discovery of AZD2716: a novel, potent secreted phospholipase A2 (sPLA2) inhibitor for the treatment of coronary artery disease*
9:30-9:50	FAR OR01 – Tracey Pirali, Università del Piemonte Orientale *Discovery of Store-Operated Calcium Entry modulators as an effective treatment for calcium-related rare genetic diseases*
9:50-10:10	FAR OR02 – Claudia Spatari, Università della Calabria *A new generation of dihydropyridines: photodegradation and photostabilization strategies*
10:10-10:30	FAR OR03 – Andrea Carotti, Università di Perugia *In Silico Approaches Supporting Pharmaceutical Analysis Enigmas*
10:30-11:00	Coffee Break
Chairpersons: Roberto Di Santo, Maria Laura Bolognesi	
11:00-11:30	FAR KN02 – Maria Laura Bolognesi, Università di Bologna *Sustainable drug discovery for neglected infectious diseases: the case of cardanol-based anti-trypanosomatid hybrids*
11:30-11:50	FAR OR04 – Rolando Cannalire, Università di Perugia *2,2-Dioxido-2,1-benzothiazines as new allosteric inhibitors of DENV NS5 RNA-dependent RNA polymerase*
11:50-12:10	FAR OR05 – Mattia Mori, Università di Siena – Istituto Italiano di Tecnologia (IIT) *Structure-based identification of HIV-1 nucleocapsid protein inhibitors active against wild-type and drug-resistant HIV-1 strains*
12:10-12:30	FAR OR06 – Tommaso Felicetti, Università di Perugia *Improvement of Staphylococcus aureus NorA efflux pump inhibition by methoxy group introduction on 2-phenylquinoline core*
12:30-12:50	FAR OR07 – Iuni Margaret Laura Trist, Università di Parma *Blocking PA-PB1 Protein-Protein Interaction with the Aid of Molecular Modelling to Counteract Influenza A Virus*
13:00-14:00	Intervallo Pranzo – Lunch Break

	Sala Paestum B
14:00-15:00	*Sessione Poster 1 (FAR PO01 – FAR PO21)*

	Sala Saturno
Chairpersons: Cosimo Altomare, Ersilia De Lorenzi	
15:00-15:30	FAR KN03 – Ersilia De Lorenzi, Università di Pavia *Development and chromatographic evaluation of Molecularly Imprinted Polymers for the selective recognition of drugs*
15:30-15:50	FAR OR08 – Michele Bianchi, Università del Piemonte Orientale *Quantitative in vivo evaluation by LC-ESI-MSn analysis of adenosine 5'-tetraphosphate (Ap4), a nucleotide related to nicotinamide phosphoribosyltransferase activities (NAMPT)*
15:50-16:10	FAR OR09 – Matteo Micucci, Università di Bologna *Thymus vulgaris L. essential oil in gastrointestinal diseases*

16:10-16:30	FAR OR10 – Azzurra Stefanucci, Università di Chieti-Pescara "G. D'Annunzio" *Structural modification of the β-sheet ARC repressor: design, conformational analysis and binding properties of linear and cyclic ARC mimetics*
16:30-17:00	Coffee Break
Chairperson:	
17:00-17:30	FAR MD01 – Claudiu T. Supuran, Università di Firenze (Pratesi DCF Medal)
17:30-17:45	FAR PZ01 – Agostino Bruno, Istituto FIRC di Oncologia Molecolare (DCF Prize)
17:45-18:00	FAR PZ02 – Sergio Valente, Sapienza Università di Roma (DCF Prize)
18:00-18:10	FAR PZ03 – Elisa Azzali, Aptuit Verona (DCF Best PhD Thesis Award)
18:10-18:20	FAR PZ04 – Bruno Cerra, Università di Perugia (DCF Best PhD Thesis Award)
18:30-20:00	*Assemblea della Divisione di Chimica Farmaceutica*

Martedi' 12 Settembre 2017

	Sala Saturno
Chairpersons: Tiziano Bandiera, Maria Menichincheri	
9:00-9:30	FAR KN04 – Maria Menichincheri, Nerviano Medical Sciences *Discovery of Entrectinib: a novel and potent inhibitor of ALK, ROS1, and Pan-TRKs kinases active in multiple molecularly defined cancer indications*
9:30-9:50	FAR OR11 – Loredana Salerno, Università di Catania *Targeting Heme Oxygenase-1 to Overcome Imatinib Resistance in Chronic Myeloid Leukemia*
9:50-10:10	FAR OR12 – Marco Lucio Lolli, Università di Torino *Potent human dihydroorotate dehydrogenase (hDHODH) inhibitors obtained by scaffold-hopping approaches: from the theoretical design to the in vivo evaluation*
10:10-10:30	FAR OR13 – Emanuele Amata, Università di Catania *Development of Sigma Receptors Nitric Oxide Photodonor Ligands with Antiproliferative Activity*
10:30-11:00	Coffee Break
Chairpersons: Federico Corelli, Rosaria Gitto	
11:00-11:30	FAR KN05 – Rosaria Gitto, Università di Messina *Discovery and optimization of isoquinoline-derived inhibitors of human Carbonic Anhydrases (hCAs)*
11:30-11:50	FAR OR14 – Giannamaria Annunziato, Università di Parma *Discovery of New, Potential Anti-Infective Compound Based on Carbonic Anhydrase Inhibitors by Rational Target-Focus Repurposing Approach*
11:50-12:10	FAR OR15 – Francesco Saccoliti, Sapienza Università di Roma *Discovery of Novel Diaryl Sulfide Derivatives as Inhibitors of Trypanothione Reductase Enzyme*
12:10-12:30	FAR OR16 – Michele Tonelli, Università di Genova *Synthesis of 4,6-diamino-1,2-dihydrotriazines as influenza viruses and respiratory syncytial virus inhibitors targeting the host DHFR*
12:30-12:50	FAR OR17 – Carmen Cerchia, Università di Napoli "Federico II" *Application of a New Scaffold Concept for the Identification of Analog Series in Commercial Databases*
13:00-14:00	Intervallo Pranzo – Lunch Break

	Sala Paestum B
14:00-15:00	*Sessione Poster 2 (FAR PO22 – FAR PO48)*

Mercoledi' 13 Settembre 2017

	Sala Paestum B
14:00-15:00	*Sessione Poster 3 (FAR PO49 – FAR PO82)*
	Sala Saturno
Chairpersons: Vincenza Andrisano, Paolo Caliceti	
15:00-15:30	FAR KN06 – Paolo Caliceti, Università di Padova *New drug delivery nanomachines: visionary concepts or reality*
15:30-15:50	FAR OR18 – Marco Paolino, Università di Siena *π-Stacked Polymers in Drug Delivery Applications*
15:50-16:10	FAR OR19 – Paola Russo, Università di Salerno *Clarithromycin dry powders for inhalation: A focus on drug solubility*
16:10-16:30	FAR OR20 – Francesco Peri, Università di Milano-Bicocca *Synthesis and preclinical evaluation of glycolipid-based TLR4 modulators: new therapeutics for inflammatory and autoimmune diseases*
16:30-17:00	Coffee Break
Chairpersons: Gianluca Sbardella, Gilberto Spadoni	
17:00-17:30	FAR KN07 – Gilberto Spadoni, Università di Urbino *Strategies to maximize therapeutic opportunities for melatonin derivatives*
17:30-17:50	FAR OR21 – Leonardo Pisani, Università di Bari *Nitrate-ester prodrugs of dual AChE-MAO B inhibitors as anti-Alzheimer Multitarget Hybrids*
17:50-18:10	FAR OR22 – Francesca Spyrakis, Università di Torino *Discovering new casein kinase 1d inhibitors with innovative MD-integrated virtual screening*
18:10-18:30	FAR OR23 – Letizia Crocetti, Università di Firenze *Isoxazol-5(2H)-one: a new scaffold for potent human neutrophil elastase (HNE) inhibitors*
18:30-18:50	FAR OR24 – Angelica Mazzolari, Università di Milano *Modelling of Glucuronidation Reactions in the MetaQSAR Database: Successful Strategies to Handle Unbalanced Data in Metabolism Prediction*

Medaglie e Premi della Divisione di Chimica Farmaceutica

Medaglia Pratesi

Claudiu T. Supuran, Università di Firenze

Premi alla Ricerca

Premio della Divisione di Chimica Farmaceutica

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Premio miglior tesi di Dottorato, Divisione Chimica Farmaceutica

Elisa Azzali, Aptuit Verona

Bruno Cerra, Università di Perugia

Keynote

- **FAR KN01** – Fabrizio Giordanetto, D E Shaw Research, "*Fragment-based discovery of AZD2716: a novel, potent secreted phospholipase A2 (sPLA2) inhibitor for the treatment of coronary artery disease*".
- **FAR KN02** – Maria Laura Bolognesi, Università di Bologna, "*Sustainable drug discovery for neglected infectious diseases: the case of cardanol-based anti-trypanosomatid hybrids*".
- **FAR KN03** – Ersilia De Lorenzi, Università di Pavia, "*Development and chromatographic evaluation of Molecularly Imprinted Polymers for the selective recognition of drugs*".
- **FAR KN04** – Maria Menichincheri, Nerviano Medical Sciences, "*Discovery of Entrectinib: a novel and potent inhibitor of ALK, ROS1, and Pan-TRKs kinases active in multiple molecularly defined cancer indications*".
- **FAR KN05** – Rosaria Gitto, Università di Messina, "*Discovery and optimization of isoquinoline-derived inhibitors of human Carbonic Anhydrases (hCAs)*".
- **FAR KN06** – Paolo Caliceti, Università di Padova, "*New drug delivery nanomachines: visionary concepts or reality*".
- **FAR KN07** – Gilberto Spadoni, Università di Urbino, "*Strategies to maximize therapeutic opportunities for melatonin derivatives*".

Fragment-based discovery of AZD2716: a novel, potent secreted phospholipase A2 (sPLA2) inhibitor for the treatment of coronary artery disease

Fabrizio Giordanetto[a]

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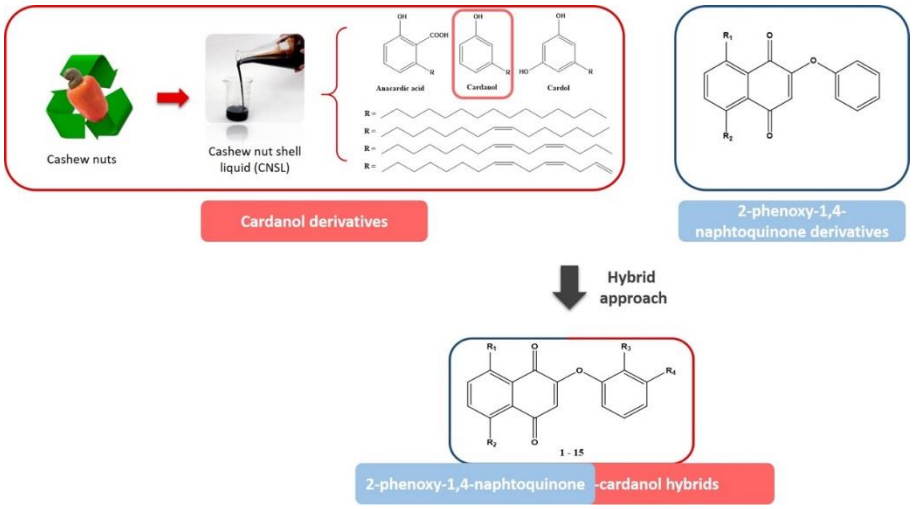
Starting from a benzamide-containing hit identified through fragment screening, a rapid medicinal chemistry campaign enabled the design of AZD2716 as a novel, potent secreted phospholipase A2 (sPLA2) inhibitor. Data-driven structure-based reasoning coupled with physicochemical parameters control resulted in the successful optimization of the efficacy, pharmacokinetic and toxicological profile of the series, culminating in the selection of AZD2716 as a clinical candidate for the treatment of coronary artery disease.

Sustainable drug discovery for neglected infectious diseases: the case of cardanol-based anti-trypanosomatid hybrids

Maria Laura Bolognesi[a]

[a] *Department of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna, Via Belmeloro, 6, 40126 Bologna, Italy*

Trypanosomatid infections are a group of highly debilitating and potentially fatal neglected diseases with major impacts on human health. Although they mainly affect populations living in poverty, with poor access to health services, leishmaniasis, Chagas disease and human African trypanosomiasis are increasingly becoming a concern for Europe too. Current insufficient chemotherapy regimens mostly rely on single-target drugs, which very often suffer by toxic side effects, lack of efficacy, and development of resistance. Moreover, cost of treatments is too high for the affected population, and the availability of quality medicinal agents on a sustainable basis is an increasingly appreciated public health care concept.[1] On this basis, efforts to lower the costs of therapy by developing new drugs based on inexpensive resources (e.g. food waste products) has gained growing attention.
Based on the above considerations, as well as on our continuous interest in multi-targeted compounds,[2] we turned our attention to cashew nut shell liquid (CNSL) as a sustainable starting material for the development of new hybrid drugs against Trypanosomatid infections. CNSL, produced in the cashew nut processing process as a waste, is a mixture of anacardic acid, cardanol, and cardol, whose structures offer opportunities for chemical derivatization. In particular, following a framework combination strategy, new hybrids have been designed by merging the naphthoquinone moiety of previously discovered anti-trypanosomatidic hits, with the phenoxy group of cardanol.



The synthesized molecules have been characterized for their anti-trypanosomal activity, both in enzyme assays and in *in vitro* parasite cultures. Given the profile of the starting hybrids, inhibition of glyceraldehyde-3-phosphate-dehydrogenase and trypanosome alternative oxidase has been studied for selected compounds. Mechanistic studies directed at elucidating the mitochondrial mechanism of action have been performed. Thanks to an effective multifaceted anti-trypanosomal profile, the current series emerge as low-cost, accessible hit compounds that deserve further characterization.

References: 1. Renslo A. R. & MacKerrow J. H. *Nat. Chem. Biol.* **2006**, 2, 701 – 710. 2. Pieretti, S. et al *PLOS Negl. Trop. Dis.* **2013**, 7, e2012; Prati, F. et al *J. Med. Chem.* **2015**, 58, 6422–34. Bruno S et al. *Chem Biol. Drug Des.* **2017**, doi: 10.1111/cbdd.12941.

Development and chromatographic evaluation of Molecularly Imprinted Polymers for the selective recognition of drugs

Ersilia De Lorenzi

Department of Drug Sciences, University of Pavia, Viale Taramelli 12, Pavia, Italy; ersidelo@unipv.it

By Molecular Imprinting Technology one can synthesize polymeric artificial receptors known as Molecularly Imprinted Polymers (MIPs) (1). In the non covalent approach, functional monomers are arranged around a molecular template (print molecule) in an appropriate solvent; then this assembly is copolymerized in the presence of an excess of cross-linker and free radical initiator, to obtain a polymeric rigid structure. Removal of the template leaves behind cavities which are complementary in size, shape and chemical functionality to the template molecule. Owing to their high physical stability, straightforward preparation, remarkable robustness and low cost, MIPs specifically designed to recognise bioactive molecules have received widespread attention and gained popularity in many fields, including purification by solid phase extraction, chiral separation, drug delivery, artificial antibodies and chemo/biosensing (2).

Preliminary evaluation and characterization of these materials is a key step before further selection and optimisation. It may conveniently include, along with advanced physical techniques, both zonal and frontal chromatography, by comparing results obtained on MIP and NIP-packed columns where NIP are synthesized in the same fashion but with the omission of the template. A wealth of information can be obtained on selectivity, loading capacity, aqueous compatibility, efficiency and reproducibility. In particular frontal analysis chromatography is an extremely powerful technique for a quantitative study of the interactions between solutes (template) and a stationary phase (MIP), as it affords the number of classes of sites on the polymer surface, saturation capacity as well as the binding constant of template associated to each class of sites.

The design and chromatographic characterisation of MIPs for the selective recognition of folic acid, methotrexate and structural analogues, bupivacaine as well as for the class-selective recognition of glucuronides will be presented. To overcome intrinsic weaknesses associated to MIPs (poor aqueous compatibility, non specific adsorption, slow mass transfer) special imprinting strategies have been implemented. The substructure or epitope approach and stoichiometric imprinting demonstrate the analogy between biological and synthetic receptors. Different MIP formats such as classical bulk particles, microparticles, capillary monoliths and composite silica-MIP particles will be evaluated for HPLC, capillary electrochromatography (CEC) and solid phase extraction (SPE) applications. Finally, the presentation will also include one of the first examples of MIP as a valid alternative to immunoassays for protein detection, to be used as biomarker discovery tool (3-5).

References: 1. Sellergren, B. (Ed.), Molecularly imprinted polymers. Man-made mimics of antibodies and their application in analytical chemistry (Techniques and instrumentation in analytical chemistry, vol.23), Elsevier, Amsterdam, 2001. 2. Chen, L. et al., J. Chem.Soc.Rev., 45 (2016) 2137. 3.Quaglia, M. et al., J.Am.Chem.Soc., 123 (2001) 2146. 4. Emgenbroich, M. et al., Chem.Eur.J., 14 (2008) 9516. 5. Ambrosini, S., et al., Analyst, 137 (2012) 149.

Discovery of Entrectinib: a novel and potent inhibitor of ALK, ROS1, and Pan-TRKs kinases active in multiple molecularly defined cancer indications

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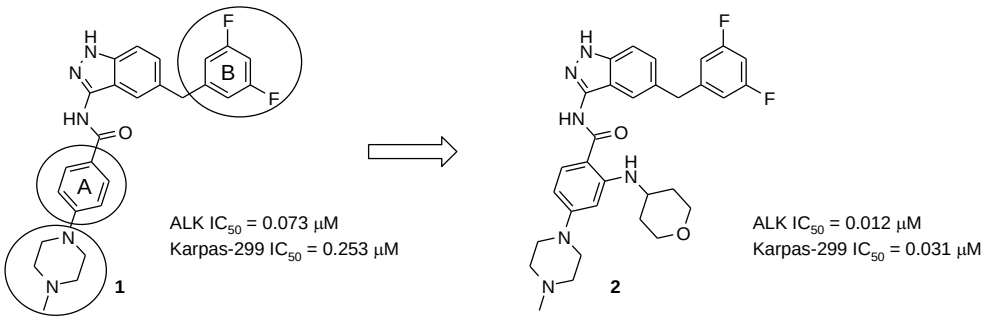
The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that plays a key role in the development of different tumor types. For instance the oncogenic protein NPM-ALK was originally identified as responsible for a subset of Anaplastic Large Cell Lymphoma (ALCL), a rare type of non-Hodgkin lymphoma (1). Subsequently and most importantly subsets of Non-Small Cell Lung Cancer (NSCLC) have been reported to be dependent on activated forms of ALK, the most frequent being the EML4-ALK protein (2). Interestingly oncogenic forms of the strictly related c-ros Oncogene 1 kinase (ROS1), and tropomyosin receptor kinase A (TRKA) have been found in the same tumor indication (3,4). In addition TRKs fusion proteins have been also identified in subsets of colorectal carcinoma (CRC) (5) and in other tumor types (6). Despite the remarkable clinical activity of the ALK inhibitor Crizotinib, the emergence of resistance mutations and of brain metastasis often cause patient relapse (7). In the search of novel and potent ALK inhibitors, the high-throughput screening (HTS) of our corporate compound collection allowed us to identify the 3-aminoindazole compound **1** (figure 1), endowed with good biochemical potency against ALK (IC_{50} = 0.073 μM) and good antiproliferative activity on the ALK-dependent ALCL Karpas-299 cell line (IC_{50} = 0.253 μM) (8, 9). From this starting point a medicinal chemistry effort, focused on the variation of ring A and ring B substitution, led to the final candidate compound **2** (entrectinib), that potently inhibits the ALK kinase (IC_{50} = 0.012 μM), and the proliferation of the ALK-dependent Karpas-299 cell line (IC_{50} = 0.031 μM).

Figure 1



Entrectinib is characterized by good oral bioavailability in all animal species, excellent *in vivo* efficacy in ALK-driven tumor models, efficient penetration of the blood-brain barrier (BBB) and good antiproliferative activity on Ba/F3 cell line transfected with different mutated forms of EML4-ALK. Moreover compound **2** is a potent inhibitor of the closely related tyrosine kinases ROS1 and TRKs, and is highly efficacious in *in vivo* related tumor models. Entrectinib is currently undergoing Phase II Clinical Trials for the treatment of selected patients affected by ALK-, ROS1-, and TRK-positive tumors.

References: 1. Shaw, A. T.; Engelman, J. A. *J. Clin. Oncol*. **2013**, *31*, 1105-1111. 2. Soda, M. *et al*. *Nature* **2007**, *448*, 561-566. 3. Bergethon, K. *et al*. *J. Clin. Oncol.* **2012**, *30*, 863-870. 4. Vaishnavi, A. *et al*. *Nat. Med.* **2013**, *19*, 1469-1472. 5. Ardini, E. *et al*. *Mol. Oncol.* **2014**, *8*, 1495-1507. 6. Amatu, A. *et al*. *Esmo Open*, **2016**; 1: e000023. 7. Gainor, J. F, et al. *J. Thor. Oncol.* **2013**, *8*, 1570-1573. 8. Menichincheri, M. *et al*. *J. Med. Chem.* **2016**, *59* (7), 3392–3408. 9. Ardini, E. *et al*. *Mol. Cancer Ther*. **2016**, *15* (4), 628-639.

Discovery and optimization of isoquinoline-derived inhibitors of human Carbonic Anhydrases (hCAs)

Rosaria Gitto[a], *Laura De Luca*[a], *Stefania Ferro*[a], *Maria Rosa Buemi*[a], *Pavlina Řezáčová*[b], *Giuseppina De Simone*[c], *Claudiu T. Supuran*[d]

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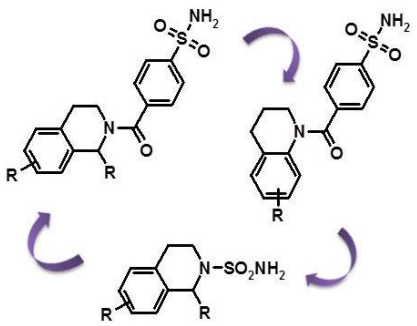
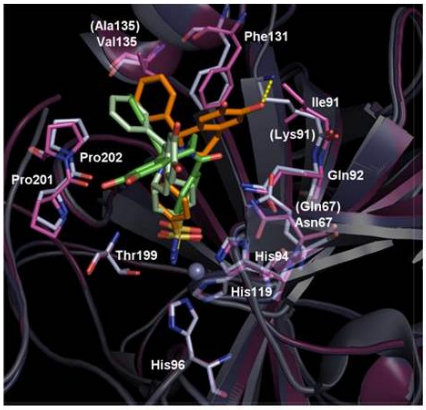
Human Carbonic Anhydrases (hCAs, EC 4.2.1.1) catalyze the reversible hydration of carbon dioxide and are involved in various physiological processes (gluconeogenesis, lipogenesis, and ureagenesis). However, their abnormal levels or activities have been often associated with several diseases. Selected CA isoforms (hCA VII, hCA IX, hCA XII and hCA XIV) have become relevant targets for the design of inhibitors for the treatment of cancer, epilepsy, obesity, glaucoma, and so on. Although the first generation of CA inhibitors (CAIs) were able to bind druggable isoforms, they were also great inhibitors of the ubiquitous hCA I and hCA II isoforms, thus displaying many undesired side-effects. Consequently, many research efforts have been recently dedicated to design new CAIs targeting hCA VII, hCA IX, hCAXII and hCA XIV. It is well-known that the (hetero)aryl-sulfonamide-based CAIs bind the catalytic zinc ion through the deprotonated nitrogen of the sulfonamide moiety; whereas, the remaining molecular fragment interacts with hydrophobic/hydrophilic residues which delimit the CA-catalytic site thus eliciting isoform selectivity.

Starting from the first series of isoquinoline-based sulfonamides CAIs (1), we performed the hit optimization for this class of compounds thus identifying selective agents toward hCA VII, hCA IX isoforms (2,3).





On the basis of cocrystal structures of hCA II in complex with the most active/selective inhibitors we further designed and synthesized isoquinoline/quinoline sulfonamides and investigated the main structure-activity relationships (4,5). To in-depth study the CA isoform selectivity we also performed molecular studies and docked the best active inhibitors in to the catalytic pocket of druggable isoforms. These studies revealed that the isoquinoline nucleus promotes extensive interactions in the active site and tunes the isoform selectivity profile.

References: 1. Gitto, R.; Agnello, S.; Ferro, S.; De Luca, L.; Vullo, D.; Brynda, J.; Mader, P.; Supuran, C. T.; Chimirri, A. *J. Med. Chem.* **2010,** 53, 2401-2408. 2. Gitto, R.; Agnello, S.; Ferro, S.; Vullo, D.; Supuran, C. T.; Chimirri, A. *ChemMedChem* **2010,** 5, 823-826. 3. Gitto, R.; Damiano, F. M.; De Luca, L.; Ferro, S.; Vullo, D.; Supuran, C. T.; Chimirri, A. *Bioorg. Med. Chem.* **2011,** 19, 7003-7007.4. Buemi, M. R.; De Luca, L.; Ferro, S.; Bruno, E.; Ceruso, M.; Supuran, C. T.; Pospíšilová, K.; Brynda, J.; Řezáčová, P.; Gitto, R. *Eur. J. Med. Chem.* **2015,** 102, 223-232. 5. Bruno, E.; Buemi, M.R.; Di Fiore, A.; De Luca , L.; Ferro, S.; Angeli, A.; Cirilli, R.; Sadutto, D.; Alterio, V.; Monti, S.; Supuran, C.T.; De Simone, G.; Gitto, R. *J. Med. Chem.* **2017**, 60, 000, DOI: 10.1021/acs.jmedchem.7b00264.

New drug delivery nanomachines: visionary concepts or reality

Paolo Caliceti, Stefano Salmaso, Francesca Mastrotto, Chiara Brazzale, Anna, Balasso, Alessio Malfanti

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Over the past years, multifunctional nanotechnology has emerged as a novel approach to overcome the biopharmaceutical pitfalls of old and new drugs, including oligonucleotides and peptides, and optimize their therapeutic performance. As a result, last generation delivery nanosystems are capable of complex functions, which enable sequential overcoming of multiple biobarriers following a certain time/site determined "logic" of events. These nanocarriers provide longer drug circulation times, higher tolerability, and site specific delivery, factors that result in better patient outcomes. Cancer represents the field of medicine application to which multifunctional nanotechnology has made the most prominent contributions. Main strategies for tumour targeting involve the exploitation of the peculiarities of the cancer tissues and cells, which include the high angiogenesis and blood vessel permeability and low lymph derange (EPR effect), the expression of specific cell membrane receptors (biorecognition) and the unique local environmental physical features (temperature, pH, redox potential and enzyme composition). Natural and synthetic polymers are landmark materials for production of novel smart nanomedicines for anticancer drug delivery. Multivalent, amphiphilic and stimuli responsive polymers have been in fact exploited to produce drug bioconjugates and self-assembling colloidal systems or to bestow peculiar physicochemical and biological properties on inert colloidal scaffolds. Stimuli sensitive polyacrylates represent unique functional modules for drug delivery as they can be used to produce assemblies, namely temperature or pH sensitive micelles and polymersomes. According to their physicochemical features, these systems can be sharply designed to dispose in the tumour tissue where the specific local conditions can induce structural changes that selectively release the drug or provide for the intracellular delivery of the drug cargos.

Polyacrylate copolymers formed by A-B blocks bearing ionisable phenol pendant units (block A) and hydrophilic neutral pendant moieties (block B) have been shown to form micelles or polymersomes depending on the ionisable/hydrophilic composition. These vesicles have been shown to efficiently deliver either hydrophobic or hydrophilic drugs yielding high cell up-take under the typical conditions of the tumour tissue. Environmentally stimuli materials can be also combined with targeting agents and cell up-take enhancers to generate sophisticate supramolecular combinations with unique in vivo performance. A-B-C triblock copolymers containing ionisable polyhistidine units (block B) and neutral hydophylic terminal blocks (block A and C) that form polymersomes in the presence of oligonucleotide drugs have been functionalized with folic acid for active cancer cell targeting. Gold nanoparticles decorated with thermosensitive polyacrylates have been found to gain switchable properties: particle aggregation and cell interaction and internalisation. The combination of the temperature and pH sensitive polyacrylate decorated nanoparticles with targeting agents has been found to bestow switchable recognition properties on the colloidal systems that can be exploited for surface recognition or cell targeting and may be used for theragnostic applications. Finally, gold nanoparticles and liposomes simultaneously decorated with pH sensitive polyacrylates and targeting agents and cell penetration enhancers have been designed to program a hide/reveal behaviour. These systems have been found to maintaining their stealth properties under physiological conditions while in the tumour tissue they reveal the cell-penetration modules that promote the cell up-take and intracellular drug delivery.

In conclusion, based on a deep knowledge of biological aspects of tumours nanotechnology offers a variety of opportunities to ameliorate the selectivity and therapeutic activity of anticancer drugs. Nonetheless, despite the development of these nanomedicines for tumour targeting are carefully in silico designed their behaviour is often unpredictable.

Strategies to maximize therapeutic opportunities for melatonin derivatives

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The neurohormone melatonin (MLT) is involved in several (patho)physiological processes including sleep, depression, anxiety, pain perception, cancer and neurodegenerative diseases (1). MLT has a pleiotropic mechanism of action as it displays antioxidant effects, activates membrane receptors and interacts with intracellular mediators such as the transcription factor Nrf2 and the MT_3 binding site (quinone reductase 2). These effects have formed the basis for the rational design of different melatonin derivatives to maximize their therapeutic potential in a wide range of established and novel indications. In particular, different ligand-based techniques, such as pharmacophore models, QSAR, conformational constraints or molecular simplification, allowed to design and develop a high number of structurally diverse classes of melatonin receptor ligands, which are employed in the treatment of sleep disturbances and depression, or are under development for novel therapeutic applications.

In this presentation, we report the design of melatonin membrane receptor ligands, based on the characterization of their pharmacophore elements and of their conformational space. Different substitution patterns allowing occupation of specific regions at the binding site have led to compounds selective for each of the two *G-protein coupled melatonin receptor* subtypes, MT_1 or MT_2 (2,3). These selective MLT receptor ligands displayed interesting sleep-inducing, antinociceptive or anxiolytic properties (4,5).

Recent investigations have also illustrated the potential for drug combination strategies to widen and further enhance the therapeutic opportunities. Information gained from pharmacophore and receptor/enzyme models has been applied to the design and optimization of multi-target compounds, which combine the interesting properties of melatonin receptor ligands with other, potentially synergistic, pharmacological activities.

Melatonin has also shown receptor-independent actions, mainly related to its radical scavenging ability and enhancement of antioxidative defense systems. These effects are evaluated for many therapeutic applications, for example, in neurodegenerative pathologies, cancer treatment, or to counteract skin aging. In this context, we designed a series of melatonin derivatives linked to ROS-responsive arylboronate triggers to investigate their potential cytoprotective activities against H_2O_2-induced oxidative damage.

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Comunicazioni Orali

Discovery of Store-Operated Calcium Entry modulators as an effective treatment for calcium-related rare genetic diseases

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Store Operated Calcium Entry (SOCE) is the major route of replenishment of intracellular Ca^{2+} in response to depletion of Ca^{2+} stores in the endoplasmic reticulum (ER). The key molecular components of SOCE machinery are STIM proteins, which function as endoplasmic reticulum calcium sensor, and Orai channels.(1)

Recently, several human diseases have been associated with mutations in these two proteins: loss-of-function mutations result in SCID-like immunodeficiencies, while gain-of-function mutations cause Stormorken syndrome, York platelet syndrome and tubular aggregate myopathy (TAM).(2) These pathologies are rare diseases with an estimated prevalence of 1 every 500 births and are currently without therapy.

Due to the recent discovery of STIM and Orai proteins, structural information is poor and only a low resolution crystal structure of Orai from *Drosophila melanogaster* has been described.(3) Therefore, the search for SOCE modulators perfectly suited to a click chemistry approach. Starting from the structure of known pyrazole derivatives (BTP, Pyr),(4) a library of candidates was designed and synthesized. Screening was performed by calcium microfluorography in wild type and mutated human embryonic kidney (HEK-293T) cells and led to the identification of both SOCE activators and inhibitors (Figure 1). Selected compounds were further evaluated by electrophysiological experiments and by *ex vivo* studies on muscle biopsies from patients affected by TAM.(5)

Chemical synthesis, metabolic stability profile and biological evaluation of this class of compounds will be discussed.

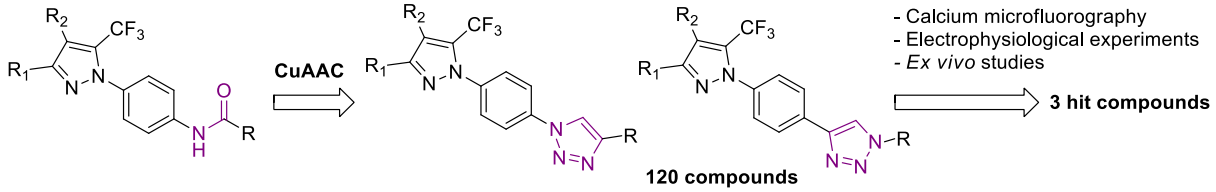

Figure 1

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A new generation of dihydropyridines: photodegradation and photostabilization strategies

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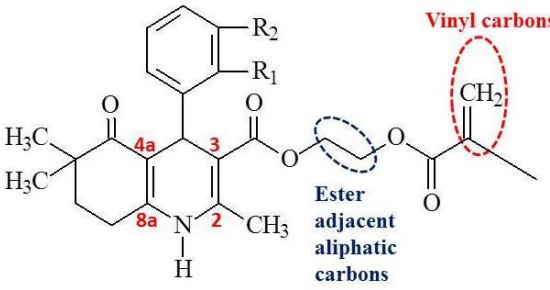
1,4-dihydropiridine derivates (DHPs) are used in the treatment of the hypertension and angina as L-type calcium channel blockers. Exposure of these molecules to natural or artificial light leads to a significant production of singlet oxygen, superoxide, or both of them, which in most cases are responsible of photosensitive/phototoxic effects (1). In a previous study (2), a quantitative structure-property relationships (QSPR) model, correlating the light sensitivity against theoretical molecular descriptors, was developed for a set of 1,4-dihydropyridine drugs. The influence of different substituents on both benzene and pyridinic rings was evaluated in terms of hydrophobic, electronic and steric parameters.

According these results, a series of new condensed DHP analogues was synthetized by microwave irradiation method. The muscle relaxant activity was evaluated and compared with that of nifedipine. All the synthesized compounds were subdued to photodegradation tests, in accordance with the ICH international rules (3). Concentration of parent compounds and by-products was calculated by multivariate curve resolution - alternating least squares (MCR-ALS) applied to the spectral data. The kinetic degradation parameters of all compounds were calculated and all the DHPs photoproducts estimated by MCR-ALS (4). Because of their well-known instability to light, several studies have been also proposed or are under investigation for producing formulations able to provide a valid photoprotection for this class of drugs. In recent years, supramolecular systems have been proposed as a means to increase the stability of drugs to light and many studies have reported significant results (5). In particular, liposomes and cyclodextrins have shown the most promising results due to their ability to improve aqueous solubility, chemical stability and bioavailability for several drug molecules by incorporating them in their core.



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In silico approaches supporting pharmaceutical analysis enigmas

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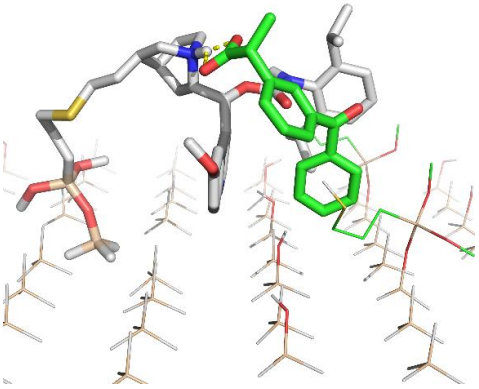
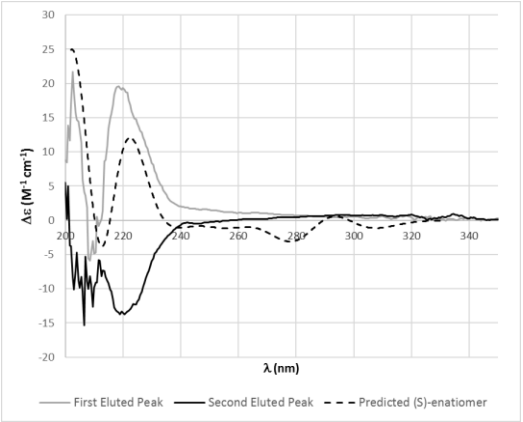
The determination of the enantiomeric elution order (EEO) is a key issue in chiral HPLC analysis. The knowledge of the absolute configuration (AC) of the more- and the less-retained enantiomer in a chiral chromatography environment is of primary importance for several reasons.
First of all, it allows organic and medicinal chemists to quickly evaluate the outcome of an enantioselective synthesis procedure (often measured in terms of enantiomeric excess value). Furthermore, in preparative chromatography applications it allows analytical and medicinal chemists to properly correlate the AC of a definite compound with one or more of its observed or measured properties (such as a specific biological activity).
Also importantly, from a theoretical point of view, understanding the fine mechanism governing the EEO means understanding the network of interactions and perturbations responsible for the stereoselective analyte (selectand, SA)-selector (SO) binding association in a definite asymmetric setting.
Chemoinformatic procedures as well as molecular mechanics and quantum chemistry techniques can be successfully applied to address chirality related problems especially when enantiomerically pure reference standards are missing. (1-3)
A number of methods developed in our laboratories to explain the mechanism of enantioselective recognition and hence to rationalize and even foresee the EEO of pharmaceutically relevant compounds in chiral chromatographic settings characterized by either low- or high-molecular weight SOs will be presented.





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2,2-Dioxido-2,1-bentothiazines as new allosteric inhibitors of DENV NS5 RNA-dependent RNA polymerase

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Dengue and the other flaviviruses are (re)emerging pathogens that are rapidly spreading from tropical to other areas of the World (1). Flavivirus infections cause flu-like symptoms that may evolve toward severe and sometimes fatal conditions (1). Furthermore, no drugs are available against these viruses (2). Targeting the viral NS5 RNA-dependent RNA polymerase (RdRp) may represent an attractive strategy to find antiflavivirus drugs (3). However, few anti-NS5 RdRp chemotypes have been reported and often they are devoid of antiviral activity in cells; moreover, no inhibitors are currently in clinical development.

With the aim to identify new NS5 RdRp inhibitors, we decided to re-task our in-house HCV NS5B inhibitors focused library (Figure 1). Representative compounds for the different chemical families were screened in vitro against Dengue 3 NS5 RdRp and the 2,2-dioxido-2,1-bentothiazines resulted promising hits with IC_{50} ranging from 11 to >50 µM. Biochemical evaluation of the entire series led to the identification of derivatives **8** and **10** able to inhibit the enzyme with 0.6 and 0.9 µM, respectively. Structure-activity relationships highlighted a key role for the C-4 benzoyl group and as suitable a halosubstituted C-6 phenoxy group. Kinetic studies for representative hit **8** indicated an allosteric mechanism consistently with a mixed type of enzyme inhibition. In agreement with the biochemical data, the predicted binding modes of representative molecules confirmed the key contribution of the benzoyl and the phenoxy regions for the binding at the so-called N pocket of the RdRp thumb domain. Unfortunately, compounds **8** and **10** were not active against DENV and other flaviviruses in cells. Thus, we speculated that modest cell permeability coupled with an ex vivo low stability of the benzoyl ester could explain the lack of antiviral activity.

However, few anti-DENV RdRp chemotypes are known and most of them are devoid of antiviral activity in cells. Therefore, the results obtained in this work indicated the 2,2-dioxido-2,1-bentothiazine scaffold as promising anti-DENV RdRp chemotype and the information acquired will drive future chemical optimization to provide new potent non-nucleoside NS5 RdRp inhibitors effective also in cell lines.

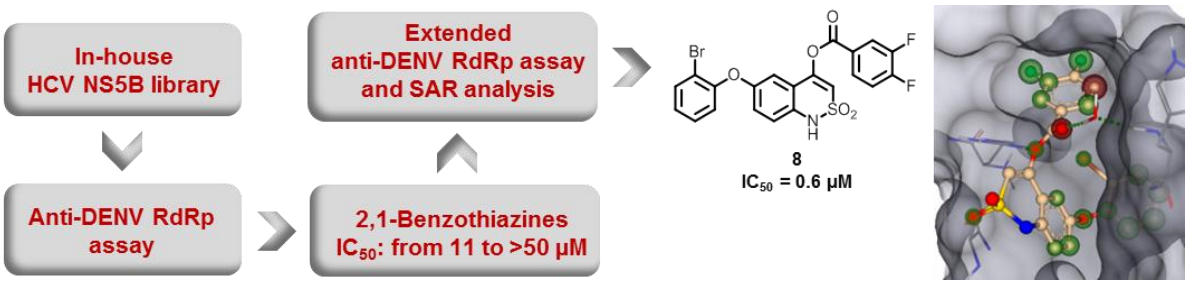

Figure 1. Workflow: from the in vitro screening of the focused library to the identification of compound 8 as potent DENV RdRp inhibitor

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Structure-based identification of HIV-1 nucleocapsid protein inhibitors active against wild-type and drug-resistant HIV-1 strains

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AIDS is still one of the leading causes of death worldwide. Current drugs that target the canonical steps of HIV-1 life cycle are efficient in blocking viral replication, but are unable to eradicate HIV-1 from infected patients.(1) Moreover, drug resistance (DR) is often associated with the clinical use of these molecules, thus raising the need for novel drug candidates as well as novel putative drug targets. In this respect, pharmacological inhibition of the highly conserved and multifunctional nucleocapsid protein (NC) of HIV-1 is considered a promising alternative to current drugs and, particularly, to overcoming DR.(2,3)

Following our research strategy, in the last eight years we devoted several efforts to targeting NC and understanding molecular determinants for its potent inhibition by different chemotypes, thus contributing to validate NC as antiretroviral target.(2,4,5,6)

Within the framework of the THINPAD project – FP7,(7) we recently established a multidisciplinary approach combining in silico screening, fluorescence-based molecular assays and cellular antiviral assays to discover non-covalent NC inhibitors. Among multiple lead compounds identified, nordihydroguaiaretic acid (NDGA) emerged as a novel natural product inhibitor of NC. By using NMR, mass spectrometry, fluorescence spectroscopy and molecular modelling, NDGA was found to act through a dual mechanism of action. First, the molecule recognizes and binds non-covalently the NC, which results in the inhibition of the nucleic acid chaperone properties of NC. In a second step, chemical oxidation of NDGA induces a potent chemical inactivation of the protein, although the binding occurs in a non-covalent manner as highlighted by mass spectrometry. Overall, the NDGA inhibits NC and the replication of wild-type and drug-resistant HIV-1 strains in the low micromolar range with moderate cytotoxicity, that makes it a profitable tool compound as well as a good starting point for the development of pharmacologically relevant NCIs.

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Improvement of *Staphylococcus aureus* NorA efflux pump inhibition by methoxy group introduction on 2-phenylquinoline core

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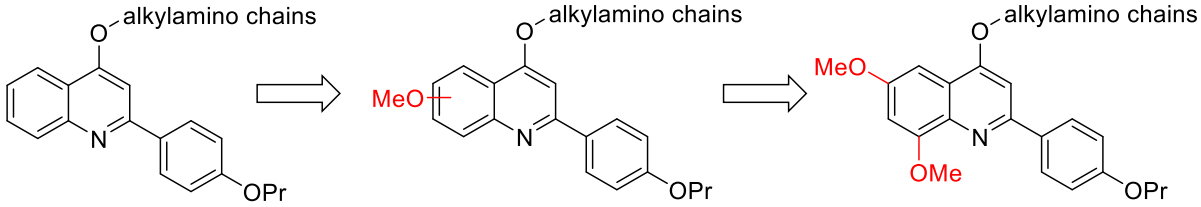
Antimicrobial resistance is nowadays a public health threat by causing several acute and chronic infections worldwide. The rapid insurgence of drug resistance in nosocomial strains is faster than the discovery of new antimicrobials having an innovative mechanism of action.(1) Thus, the strategy to sustain the antimicrobial activity of an approved antibiotic with a helper compound devoid of any antibacterial activity but having the capability to restore drug sensibility against resistant strains is taking hold. Therefore, since microbial efflux pumps are recognized as a main contributor to a basal or high level of resistance in several different microbes, to find an efflux pump inhibitor (EPI) can result an excellent strategy to restore strain sensibility to extruded antibacterials. The most expressed efflux pump in *Staphylococcus aureus* is NorA, associated with fluoroquinolone resistance and responsible for extrusion of unrelated substances out of the bacterial cell.(2)
Previously, we reported a series of 2-phenylquinoline derivatives as potent NorA EPIs.(3,4) Starting from these promising results and maintaining the best groups resulted from the preliminary SAR data, the introduction of a methoxy group, a substituent frequently recurrent in natural or synthetic NorA EPIs, was planned. Thus, new series of C-5, C-6, C-7, or C-8 (mono)methoxy-2-phenylquinoline derivatives were synthesized and tested.(5) Hence, the interesting results obtained both in terms of NorA EPI activity and synergistic activity with ciprofloxacin (CPX) against resistant *S. aureus* strains prompted us to further explore the double introduction of methoxy groups on the same core thereby affording dimethoxy-2-phenylquinoline derivatives.



Therefore, a new set of 6,8-dimethoxy-2-phenylquinoline derivatives was synthesized and tested primarily by ethidium bromide (EtBr) assays in *S. aureus* strain overexpressing *norA* gene. Finally, compounds endowed with an EtBr efflux inhibition ≥ 80 % and devoid of antibacterial activity were assayed in synergism with CPX against a panel of resistant *S. aureus* strains. Results of this study will be presented.

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Blocking PA-PB1 protein-protein interaction with the aid of molecular modelling to counteract Influenza A Virus

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A still worrying health burden responsible for important consequences on the global morbidity, mortality and economy is influenza, a seasonal disease commonly known as "flu".(1) It is caused by RNA viruses that infect vertebrates and belong to either one of the three genera of the *Orthomyxoviridae* family. Among these, influenza A is accountable for severe upper respiratory diseases in humans that occur seasonally with epidemic and sometimes pandemic proportions.(2) Anti-influenza countermeasures are available, however the existing anti-influenza vaccine needs annual updating and there is a rapid emergence of viral strains resistant to available therapy, making the need for antiviral drugs that exploit novel mechanisms of action urgent.(3) The viral RNA polymerase (RdRp) is a heterotrimer essential for viral replication and less prone to mutations than current viral targets. In particular, the interaction between two of its three subunits (PA, and PB1) is essential for RdRp activity and viral infectivity, making the disruption of this protein-protein interaction a promising drug design strategy.(4)

Through a virtual screening procedure we have identified a novel class of 3-cyano-4,6-diphenyl-pyridines that inhibit the PA-PB1 interaction.(5) In our model, these molecules bind to PA in the site of binding of PB1, superposing very well with its N-terminal residues. We chemically modified this scaffold aiming the optimization of the compounds' activity through the enhancement of interactions with PA.(6) In this presentation, the good cytotoxicity profile of the molecules and both their ability of disrupting the PA-PB1 interaction and antiviral activity will be discussed. Furthermore, the results of the study of the mechanism of action, clarified through molecular modelling simulations, will be discussed.

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Quantitative *in vivo* evaluation by LC-ESI-MS^n analysis of adenosine 5'-tetraphosphate (Ap4), a nucleotide related to nicotinamide phosphoribosyltransferase activities (NAMPT)

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Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD) synthesis, that is an essential coenzyme for maintaining the cellular homeostasis (1,2). Adenosine 5'-tetraphosphate (Ap4) is a natural nucleotide known as the most potent vasoactive purinergic mediator in mammals (3). Preliminary in vitro (4) studies have shown that Ap4 production is related to NAMPT activity. However, it has never been reported whether NAMPT can catalyze the synthesis of Ap4. The main aim of the work was to develop a new bioanalytical LC-ESI-MS^n method to quantify Ap4 in engineered B16 Melanoma cells. Secondly, to quantify, with the same method, all the analytes (adenosine 5'-diphosphate, adenosine 5'-triphosphate, nicotinamide, nicotinamide mononucleotide and NAD) involved in NAD homeostasis to better understand the two different NAMPT activities. In order to investigate NAMPT PRTase and ATPase activities, various cells lines were analyzed which differ each other for intracellular NAMPT levels. As result, intracellular Ap4 levels were increased more than two times in cells over-expressing NAMPT (v. WT cells; $p<0.05$) and were significantly reduced in cells silenced for the enzyme (v. WT cells; $p<0.05$). Moreover, WT cells treated with FK866, confirmed that it is a selective inhibitor of NAMPT PRTase activity, but not of NAMPT ATPase activity. In fact, the data collected showed a significant downregulation of NAD levels but in contrast, an upregulation of intracellular Ap4 levels (v. WT cells; $p<0.01$) (5). This indicates that both the reactions catalyzed by NAMPT should be equally considered when investigating the effect of NAMPT inhibitors.
In conclusion, the study reports that Ap4 production in melanoma cells is dependent on NAMPT expression and highlights novel mechanisms by which this enzyme could exert the plethora of actions that are attributed to it.

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Thymus vulgaris L. essential oil in gastrointestinal diseases

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In the last few years an increase in the scientific interest for essential oils has been observed (1). Recent studies have proposed their multiple effects, from direct effects on autonomic nervous system to a synergic activity with antibiotic drugs, aiming at membrane structure disruption and bacterial cell permeabilisation. The increasing emergence of drug-resistant bacteria led the research also towards the use of essential oils as potential alternatives. Moreover, some essential oils showed also antifungal properties and could represent viable therapeutic strategies addressed to drug-resistant fungal strains.

Thymus vulgaris L.

Essential oil obtained from *Thymus vulgaris* L., a perennial plant belonging to Lamiaceae family, has been known since long time for its biological effects (2). The lipophilic nature of its secondary components allows them to cross cell wall, alter membrane composition and increase membrane fluidity, leading to leakage of ions and cytoplasmic molecules.
The aim of this study is the investigation of the chemical composition of *Thymus vulgaris* L. essential oil and of its biological activities towards gastrointestinal tissues and microorganisms.
An analytical chemical profiling approach with quali-quantitative purposes was exploited to study *Thymus vulgaris* L. secondary metabolites, by means of liquid chromatography and capillary electrophoresis coupled to diode array detection and mass spectrometry (LC-MS/MS and CE-DAD).
Thymus vulgaris L. essential oil was studied towards the main pathogenic and non-pathogenic bacterial and fungal species in the gastrointestinal system. Similarly, in the guinea pig, its effects on intestinal basal and stimulated contractility were investigated. These overall preliminary results suggested that *Thymus vulgaris* L. essential oil may be useful in gastroinestinal inflammatory diseases.

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Structural modification of the β-sheet ARC repressor: design, conformational analysis and binding properties of linear and cyclic ARC mimetics

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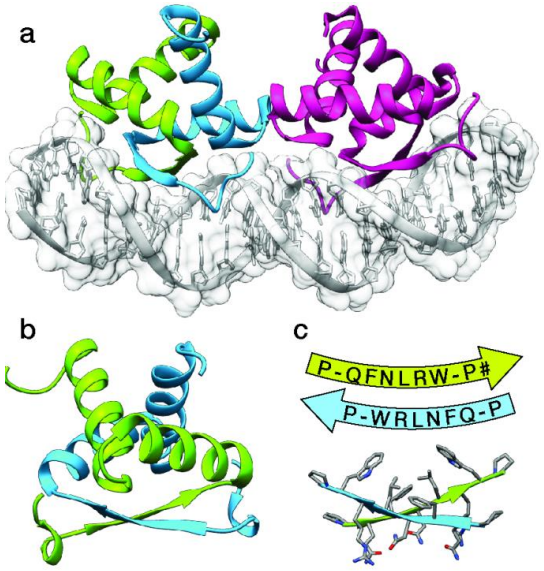
ARC repressor (apoptosis repressor with caspase recruitment domain) is an inhibitor of apoptosis critically involved in many physiological and pathological conditions (1). In human being ARC is primarily expressed into striated muscle tissue, which normally doesn't undergo a rapid cell turnover, this suggest that it may play a protective role on the muscular fibers and possible implications in the prevention against the Duchenne Muscular Dystrophy and several tumors (2). In this work we report the synthesis and binding properties of novel β-sheet and β-hairpin ARC mimetics, based on the amino acid sequence of the native β-sheet domain (3). Our data showed unspecific interactions between the novel chemical entities and the DNA sequence, providing more insights into the biomolecular recognition process and laid the groundwork for the design of novel β-sheet folded peptides as valuable substitutes of transcription factor proteins in drug's therapy.



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Targeting Heme Oxygenase-1 to Overcome Imatinib Resistance in Chronic Myeloid Leukemia

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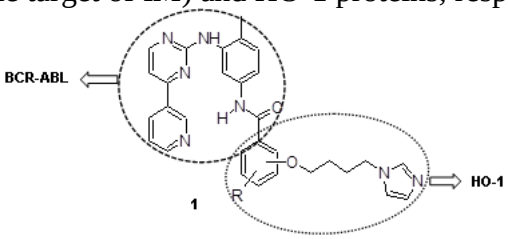
Heme oxygenase-1 (HO-1) is the enzyme catalyzing the rate-limiting oxidative degradation of cellular heme into free iron, carbon monoxide (CO) and biliverdin, which is then rapidly converted into bilirubin (1). HO-1 is considered a survival molecule in various stress-related conditions (2). By contrast, growing evidences suggest that HO-1 is a survival-enhancing molecule also in a number of solid and blood cancers, promoting carcinogenesis, tumor progression, and chemo-resistance. Chronic myeloid leukemia (CML) is currently therapeutically well treated with tyrosine kinase inhibitors (TKIs) such as Imatinib (IM) and its congeners, nevertheless resistance to all kind of current drugs persists in a number of patients. Therefore, identification of new eligible targets that may improve CML therapy is of general interest. Recent studies provided evidence that silencing HO-1 in IM resistant CML cells by siRNA resulted in induction of apoptosis, restoring IM activity (3, 4). To support these studies, we recently discovered that two novel imidazole-based HO-1 inhibitors were able to restore IM sensitivity in IM resistant LAMA-84 R cells (5). These results confirmed that inhibition of HO-1 activity can be a viable new anticancer strategy and co-administration of a HO-1 inhibitor with IM opens up new perspectives in the management of IM resistance. An alternative approach to the co-administration of two agents would be to combine multiple activities within the same compound providing a superior therapeutic effect and side effect profile compared to the action of single molecules. In this respect, conjugation of two biologically active molecules into one hybrid compound can be beneficial for the treatment of diseases with complex etiologies such as cancer (6). On these bases, the aim of this study is the design, synthesis and evaluation of antitumor properties of a new series of hybrid compounds obtained combining IM structure with our HO-1 inhibitors (**1**). These hybrids contain an IM-like portion and an aryloxyalkylimidazole moiety, needed for the interaction with BCR-ABL (the target of IM) and HO-1 proteins, respectively.



Multiple biological tests are in progress, including evaluation of HO-1 enzymatic activity, quantification of BCR-ABL, and viability of sensitive and resistant CML cell lines. Finally, in order to improve pharmacokinetic properties, reduce the undesired distribution to off target tissues, concentrate the drug in the target organ, and increase the half-life, Styrene Maleic Acid (SMA) nano-particles containing the most interesting HO-1/TKIs will be prepared. Results obtained so far will be presented at the meeting.

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Potent human *dihydroorotate dehydrogenase* (*h*DHODH) inhibitors obtained by scaffold-hopping approaches: from the theoretical design to the *in vivo* evaluation.

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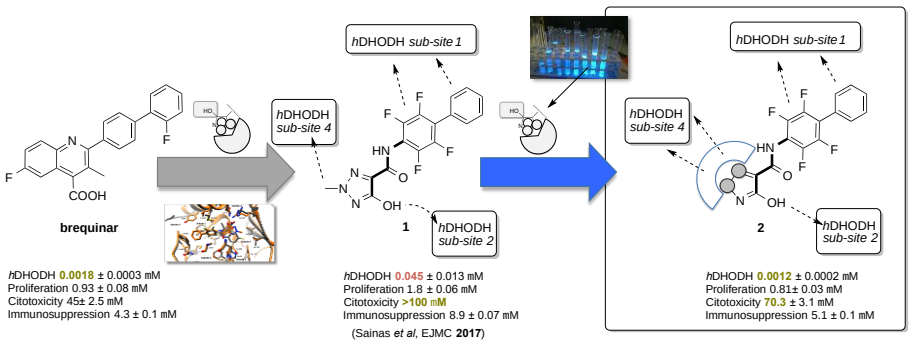
Human dihydroorotate dehydrogenase (*h*DHODH) catalyzes the rate-limiting step in the *de novo* pyrimidine biosynthesis where dihydroorotate (DHO) is converted to orotate (ORO). Being already validated as therapeutic target for the treatment of autoimmune diseases,[1] as *rheumatoid arthritis* or *multiple sclerosis*, in the fall 2016[2] *h*DHODH was associated to *acute myelogenous leukemia* (AML), a disease that has not seen a new therapies in four decades being *cytarabine* still representing the last significant advance.[3] This discovery opened a totally new prospective in *h*DHODH field.
Starting from *brequinar*, one of the most potent known *h*DHODH inhibitors, and by applying innovative *scaffold-hopping* replacement, we recently designed a new generation of potent and selective *h*DHODH inhibitors.[4] Their general structure is characterized by a biphenyl moiety joined through an amide bridge with an acidic hydroxyazole scaffold (*hydroxylated thiadiazole*, *pyrazole*, *triazole* and *furazan*). All the compounds presented nano-molar activity on the isolated *h*DHODH, just one digit from the *lead* brequinar. The best compound the series, the hydroxytriazole (**1**), also showed *in vitro* better drug-like properties.
In this occasion, we move ahead presenting a second generation of inhibitors designed by using as hydroxyazole a novel fluorescent isostere of carboxylic acid. Using a combination of *structural*- and *ligand*- optimization strategies we obtained compound **2** (see Figure), this latter able to reach brequinar *h*DHODH potency levels although using a different scaffold. Theoretical design, modeling, synthesis, SAR, fluorescent properties, X-ray crystallographic poses, biological assays (cell viability, proliferation, cytotoxicity, immunosuppression), ADME and *in vivo* preliminary experiments are here presented and discussed.



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Development of Sigma Receptors Nitric Oxide Photodonor Ligands with Antiproliferative Activity

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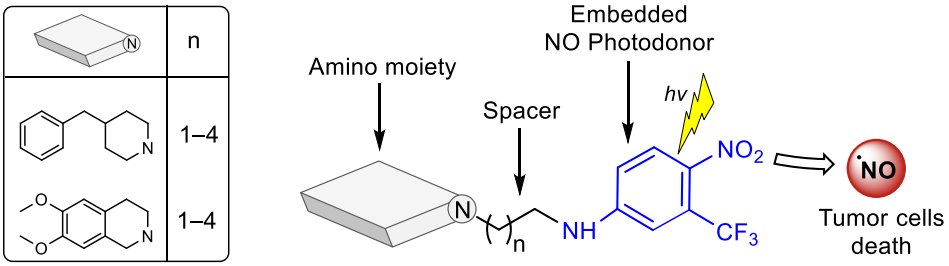
Nitric oxide (NO) is a short-lived gas with recognized important roles in various biological and physiological processes (1). Modulation of NO levels seems to have benefits in the treatment of cancer. However, due to its reactive and unstable gaseous nature, the spatiotemporally well-controlled NO exposition to cancer sites is challenging. For selective and effective delivery of cytotoxic NO, the use of photo-controllable NO donors is useful to induce a NO-dependent cellular response under light irradiation (2).
Additionally, one of the major issues of conventional anticancer drugs is the high toxicity towards proliferating cells, including normal cells (3). A strategy for minimizing this toxicity may result by conjugating the therapeutic agent with a tumor-cell-specific ligand, selectively recognized by a biological target overexpressed in cancer cells (4). Sigma (σ) receptors represent a class of proteins useful for cancer cells targeted drug delivery, being highly overexpressed in cancer cells (5).



In light of the aforementioned, we turned our interest to the combination of σ receptors chemical moieties with a NO photodonor scaffold, developing a new series of hybrid ligands. The novel compounds are made of a portion able to bind to the overexpressed σ receptors, the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline or 4-benzylpiperidine amino moieties, linked to a NO photodonor scaffold, a 4-nitro-3-(trifluoromethyl)aniline, and separated by two to five methylene unit spacers. The new synthesized compounds have been evaluated in *in vitro* σ receptor binding assays and tested for their ability to release NO under appropriate light irradiation. Based on these previous findings, best compounds were selected for dark/light *in vitro* studies on tumorigenic and non-tumorigenic cell lines variously expressing σ receptors. Preeminent results showed a significant antiproliferative activity on tumorigenic cells when photoactivated while no activity was observed in dark condition and in non-tumorigenic cells at chosen concentrations.

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Discovery Of New, Potential Anti-Infective Compound Based On Carbonic Anhydrase Inhibitors By Rational Target-Focus Repurposing Approach

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Drug-repurposing or repositioning (DR) denotes an ensemble of tasks aimed to the identification of new drug indications for existing drugs, and is an alternative strategy in drug discovery program, both in pharma and academia. In academia, DR can be also translated into compound-recycling (CR) that is the repurposing of compound library collections already available in-house. Indeed, small molecules already synthesized, that resulted inactive against a target of interest, can be tested on other targets, leading to a new-purpose for an old molecule.[1]

We embarked in a project aimed at the repurposing of the compound libraries available in-house, looking for a new potential applications for our compounds. In this scenario a rational target-based drug repurposing approach was applied.[2] The analysis of the data available in literature, for similar classes of chemical structures, allowed us to identify the Carbonic Anhydrase (CA, EC 4.2.1.1) metalloenzyme family as potential target of some of our compound series.

We proceed to the analysis of the fragments and chemotypes present in our library by applying the Maximum Common Substructure (MCS) decomposition approach. A thoroughly validated docking screenings protocol was combined with chemical synthesis[3] and in vitro assays to disclose new potential CA inhibitors. Such a method allowed us to identify eleven compounds as potential CA inhibitors (CAIs).

The compounds were, therefore, tested *in vitro* for their ability to inhibits different classes and isoforms of CA superfamily, leading to the discovery of a CAIs active in the low μM range, but characterized by: (i) two unprecedented chemotypes CAIs inhibitors; (ii) an unprecedented selectivity profile for this class of molecules, with the ability to preferentially bind microbial CAs over the human ones; (iii) good Ligand Efficiency and Binding Efficiency Indexes (BEI) with respect to that marketed CAIs. Modelling studies together with *in vitro* assays allowed us to identify new CAI chemotypes, which are characterized by a low μM affinity for microbial CA.[4] Even if, the activity profile of the compounds needs to be improved, the identified molecules can represent excellent hits to be further optimized in hits-to-lead campaigns.

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Discovery of novel diaryl sulfide derivatives as inhibitors of Trypanothione Reductase enzyme

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Trypanosomatidae protozoa are the causative agents of several tropical diseases, such as African sleeping sickness, Chagas's disease and various forms of leshmaniasis, causing millions of deaths every year mainly in the developing world.(1) Nowadays, no safe and efficacious drugs are available for the treatment of most of these neglected tropical diseases, and, furthermore, high costs and increasing number of drug-resistant pathogens render the treatment even difficult.(2,3) Therefore, there is a strong need to develop more efficient and affordable antiprotozoal compounds and identify new promising targets. In this context an innovative approach is targeting protein essential for the parasite survival but absent in the human host. Instead of the mammalian redox defense machinery based on glutathione, the trypanosomatid parasites possess trypanothione as the main defending system against oxidative damage.(4,5,6) Trypanothione (TSH_2) is kept in its reduced state by trypanothione reductase (TR), a NADPH dependent flavoprotein which acts as key enzyme of the trypanothione pathway, being critical for the protozoan survival, thus representing an attractive and promising target for the development of new potential drugs.(2,3) Furthermore, due to structural differences between the protozoan enzyme and the human homolog glutathione reductase (GR), a selective therapeutic approach might be possible. Following the discovery of some related compounds described in literature as TR inhibitors,(7) we evaluate the antiprotozoal activities of our in-house diaryl sulfide derivatives and some of the them proved to be active in whole cell assays, showing inhibitory activities within the micromolar range on different protozoa. Moreover, we found that our derivative **RDS 777** was able to inhibit TR of *L. infantum* (*Li*TR) with good efficiency, showing a Ki of 0.25 μM that is six times lower than that of Sb(III), the active form of antimonials being the most used drug against leishmaniasis.(8) Thus, we solved the X-ray structure of *Li*TR in its oxidized state in complex with **RDS 777** at 3.5 Å resolution, disclosing its mechanism of action. Indeed, this structure shows that the compound localizes at the catalytic site, engaging interactions with the residues more involved in the catalysis namely: Glu466', Cys57, Cys52 and Tyr110 thereby inhibiting the trypanothione binding. These data provide important insight that could be very helpful for future development of this class of inhibitors endowed with focused structural modifications in order to increase affinity and potency against protozoan target.

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Synthesis of 4,6-diamino-1,2-dihydrotriazines as influenza viruses and respiratory syncytial virus inhibitors targeting the host DHFR

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The *Orthomyxoviridae* and *Paramyxoviridae* families comprise important respiratory pathogens, such as, influenza viruses and respiratory syncytial virus (RSV). The acute respiratory illnesses caused by these viruses represent a major medical need (1,2). Currently used antiviral drugs preferentially inhibit virus-specific replication factors. Host-targeting antivirals represent an alternative and emerging strategy to address host proteins involved in virus life cycle. Herein, we have identified a series of cycloguanil-like derivatives able to inhibit influenza A and B virus and RSV replication targeting the host dihydrofolate reductase (DHFR) enzyme (3). The 1-aryl-4,6-diamino-1,2-dihydrotriazines (**2**-**28**) were designed by exploring the effect on biological activity as a result of the chemical variation of the *para*-Cl substituent on the phenyl ring and/or of the two methyl groups at C(2) of cycloguanil (**1**) with smaller/bulkier alkyl groups. They proved active against influenza B virus in the low micromolar range, reaching for the best compounds (**11**, **13**, **14** and **16**) the sub-micromolar potency of zanamivir (EC_{50}= 0.060 µM), and markedly exceeded (up to 327 times) the antiviral efficacy of ribavirin. Besides inhibiting two influenza A strains, more importantly the compounds displayed nanomolar activity against RSV with a SI (CC_{50}/EC_{50}) >10,000 for compounds **11**, **14** and **16** (EC_{50} ~0.008 µM), far surpassing the potency and safety profile of the licensed drug ribavirin (EC_{50}= 5.8 µM, SI>43). The interesting dual activity of these cycloguanil analogues against influenza and RSV viruses, *via* inhibition of the cellular hDHFR enzyme, points to this host factor as a new therapeutic target for these two respiratory viruses. In fact, reversal effect on antiviral activity has been demonstrated in RSV-infected HeLa cells, exposed to compound **14**, in combination with different concentrations of dihydrofolic acid, such as natural DHFR substrate. These compounds, tested against the recombinant protein of the hDHFR, also confirmed to bind this enzyme in the sub-micromolar range. Kinetic inhibition studies showed a competitive inhibition behavior, and docking studies disclosed the most probable binding mode for this class of hDHFR ligands. The possibility to suppress influenza viruses by interfering with the purine or pyrimidine pathway was proposed for a few other enzymes (4), but our study is the first to identify the relevance of host hDHFR in antiviral therapy. Therefore, we deemed interesting to further investigate the SAR of this class of compounds, exploring a novel azaspiro-4,6-diamino-1,2-dihydrotriazine scaffold different from the previous one. It was obtained by exploiting in a synthetic step the 4-piperidone, as useful building block, which allowed through its nitrogen atom to introduce an additional reactive center of molecular diversification. Within the new series, interesting hit compounds have been identified, warranting further investigations of their chemical space for the design of improved host-targeting antiviral agents.

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Application of a New Scaffold Concept for the Identification of Analog Series in Commercial Databases

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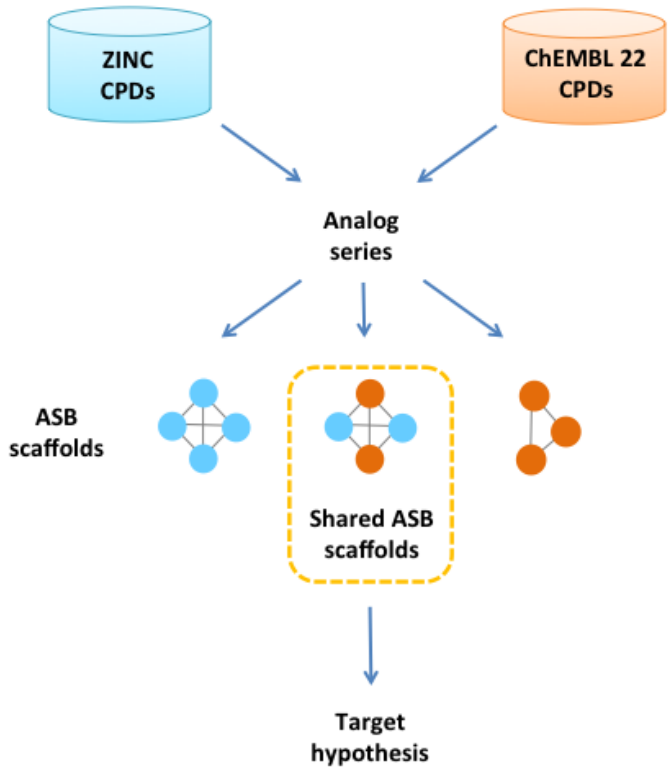
In medicinal chemistry scaffolds are used to represent core structures of compounds. (1,2) Scaffolds are intensely explored in computer-aided drug design: of particular interest is the association of core structure motifs with specific biological activities. We hereby describe our analysis to globally view accessible analog space and systematically search for analog series in large compound repositories. The analysis was focused on a recently introduced molecular scaffold definition, termed analog series-based (ASB) scaffold. (3) ASB scaffolds were designed to further increase the medicinal chemistry relevance of scaffolds by incorporating chemical reaction information. Therefore, analog series were systematically extracted from the ZINC drug-like database as well as ChEMBL 22, and the resulting ASB scaffolds were collected. Then, the ASB scaffolds shared by ZINC and ChEMBL compounds were prioritized. In this way, target annotations from ChEMBL can provide novel compound-target hypothesis. (4)



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π-Stacked Polymers in Drug Delivery Applications

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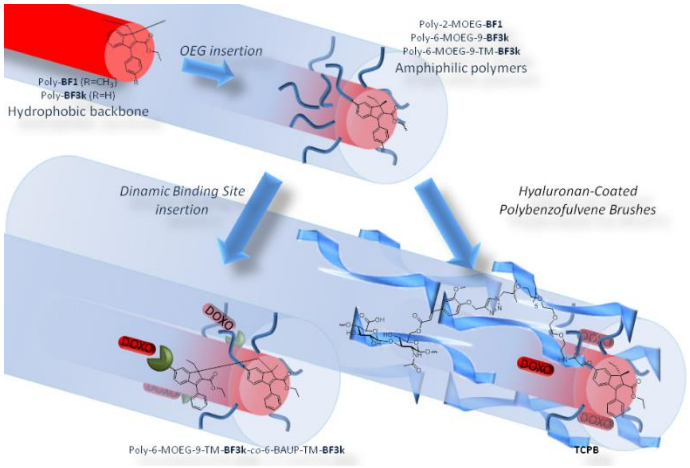
In parallel with the discovery of new bioactive compounds, research in the pharmaceutical field has targeted to the development of new formulations able to control the amount and release speed of the drugs into the organism in order to improve their therapeutic action. For this purpose, non-conventional dosage forms, commonly called drug delivery systems (DDS), have been developed. Over the last 15 years, our research group has been involved in the discovery and the application of a new class of π-stacked polymers: the polybenzofulvenes. A large variety of benzofulvene derivatives were synthesized and allowed to polymerize spontaneously by solvent removal in the apparent absence of catalysts or initiators. The polybenzofulvene derivatives are characterized by interesting features including tunable solubility in different solvents and aggregation behavior in water, and propensity to generate nanostructured aggregates. Among the large variety of structure manipulations, we explored the insertion of oligo(ethylene glycol) (OEG) side chains on the polymer backbone through different synthetic strategies to obtain polybenzofulvene molecular brushes (PBFMBs) capable to interact with the water.(1,2,3) PBFMBs have been employed to complex and release bioactive molecules, such as immunoglobulin G (IgG) from a strong physical hydrogel obtained with poly-2-MOEG-9-**BF1**(4) or the anticancer peptide leuprolide from nanogel obtained with poly-6-MOEG-9-**BF3k**(5) through non specific protein–polymer interactions.



In a subsequently strategic step, PBFMBs have been engineered with a synthetic dynamic receptors capable of interacting with the anticancer drug doxorubicin (DOXO) and delivering it to cancer cells.(6) Recently, a PBFMB has been functionalized with low molecular weight hyaluronic acid (HA) macromolecules in a tri-component polymer brush (TCPB) to develop a new advanced biomimetic functional material.(7) TCPB has been employed in the preparation of a nanostructured drug delivery system capable of deliver DOXO to cancer cells exploiting the selective interaction of the HA with the CD44 receptors.

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Clarithromycin dry powders for inhalation: A focus on drug solubility.

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Introduction:

Apart from deposition, the success of an inhalation therapy is related to the ability of the deposited drug to dissolve in the fluids lining the lung. In the case of cystic fibrosis, the thick mucus acts as a physical barrier to the dissolution of the drug, weakening the drug effectiveness. Among antibiotic therapy, macrolides are not commonly used in CF to treat infections caused by mucoid strains of *Pseudomonas aeruginosa*. Notably, several studies described a clinical benefit when macrolides were administered, with a decrease of the bacterial ability to adhere to airways epithelial cells (1). Thus, our research was focused on the design and development of a stable and effective Dry Powder Inhaler (DPI) containing an association of a macrolide antibiotic (clarithromycin, CLA) and a mucolytic agent (N-acetylcysteine, NAC).

Methods

Micronized powders were obtained from different hydro-alcoholic solutions containing 2-Propanol from 30% to 50% (v/v) and CLA and NAC in equimolar ratio, with a total powder concentration of 3% (w/v). All liquid feeds were dried using a Buchi Mini Spray Dryer B-191. Particle size of spray-dried particles was determined using a light-scattering laser granulometer equipped with a tornado powder dispersing system. The *in vitro* aerodynamic properties of the *Spray-Dried* (SD) powders were assessed be a *Single Stage Glass Impinger* (SSGI) using the monodose DPI RS01 model 7 as device to aerosolize the powders (*Eur. Phar. 8).* To study and compare the behavior of different Spray Dried (SD) powders when in contact with small amount of fluids (closer to in vivo conditions), a vertical diffusion cell equipment (Franz-type cells) was used.

Results and Discussion

The process yield increased with the 2-PrOH content, thanks to the reduction of the energy heat of the solvent mixture. Particle diameter (d_{50}) of the SD particles ranged between 2.6 μm and 3.3 μm, suitable values for inhalation. Morphology studies evidenced that the increase in 2-PrOH concentration caused the formation of spherical particles together with corrugated ones, in a blend not very homogenous. As to the aerodynamic behaviour, the produced powders showed all excellent flow and aerodynamic properties as evidenced by the very high emitted doses and fine particle fractions. Finally, compared to CLA batches (drug in non-salt form), higher dissolution profiles were obtained with CLA-NAC powders. These results confirmed that the spray drying process together with drug salification enhanced both powder solubility and wettability, with no need of potentially toxic excipients.

Conclusions

Co-spray dried powders of CLA and NAC showed good technological and aerodynamic properties, appearing as a valid pharmacological support for a better management the CF respiratory disease.

Acknowledgments

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Synthesis and preclinical evaluation of glycolipid-based TLR4 modulators: new therapeutics for inflammatory and autoimmune diseases

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Toll-like Receptor 4 (TLR4) activation by bacterial lipopolysaccharide (LPS) is the basis of inflammatory and innate immune response to invading pathogens in humans. However, excessive TLR4 activation by bacterial and endogenous ligands causes a large array of inflammatory and autoimmune pathologies. High-affinity TLR4 agonists and antagonists are therefore drug candidates to target a large array of diseases, some of which are still lacking specific pharmacological treatment. Recent achievements in the rational design, synthesis, and biological characterization of new, glycolipid-based Toll-like Receptor 4 (TLR4) modulators are reported. In the frame of the MSCA-ETN European project TOLLerant (www.tollerant.eu) we are studying the TLR4 activity of synthetic glycolipids mimicking the structure of lipid A, in the perspective to develop new TLR4-based small-molecule therapeutics (1). We are using the same molecules as high-affinity ligands of the MD-2 and CD14 co-receptors that are important players of the TLR4 activation process. With these synthetic probes we aim to dissect and study the molecular mechanisms of TLR4 activation and signaling. In particular we report on recent findings in the activity of such drug candidates to block influenza virus lethality (2), amyotrophic lateral sclerosis (ALS)(3), inflammatory bowel diseases (IBDs), aortic aneurysm (4) and other inflammatory diseases. Very recent achievements in the synthesis of non-toxic TLR4 antagonists based on different biocompatible scaffolds will be presented.
NMR binding studies, biochemical experiments with purified MD-2 co-receptor, and microscopy imaging will be presented. These recent data give new insights into the mechanism of action of synthetic, glycolipid-based TLR4 modulators.

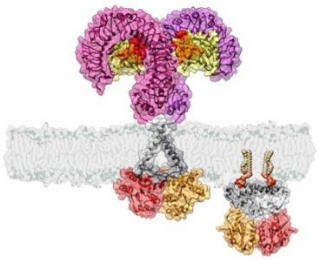

Figure 1. The TLR4/MD-2 activated heterodimer (pink and yellow) on cellular membrane.

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Nitrate-ester prodrugs of dual AChE-MAO B inhibitors as anti-Alzheimer Multitarget Hybrids

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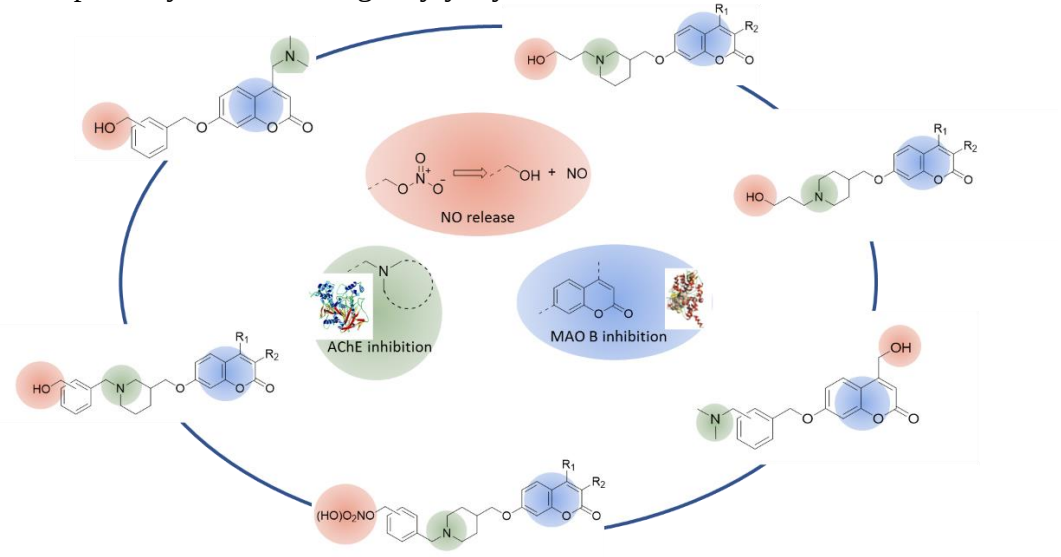
The discovery of disease-modifying agents to treat Alzheimer's disease (AD) is a challenging research topic due to the multifactorial etiopathogenesis.(1) An innovative multitarget strategy aims at identifying drugs able to modulate simultaneously two or more relevant targets in the search for additive effects ultimately curative.(2) Along this idea, herein we propose the development of compounds able to promote synergistic activities against AD as follows:

- inhibition of acetylcholinesterase (AChE), for counteracting cholinergic depletion at the synaptic level;
- inhibition of monoamine oxidase B (MAO B) in reactive astrocytes, for reducing oxidative stress arising from hydrogen peroxide activity;
- release of nitric oxide (NO), for exerting neuroprotective and precognitive actions via ERK-CREB pathways and soluble guanylyl cyclase at low fluxes.



Among the possible NO-donors, alkyl nitrate esters were chosen to investigate the potential release of alcohol-based active metabolites upon hydrolysis. In order to exploit this bioactivation reaction, different dual AChE-MAO B inhibitors bearing an hydroxymethyl group (3) were developed before being transformed into the corresponding nitrate prodrugs in the case of the most active alcohol derivatives. By following a fragment-merging approach three diverse pharmacophore features, each potentially promoting a relevant activity, were joined in multifunctional compounds, while changing the linkage pattern.(4) To this aim, a planar coumarin backbone, selected to attain MAO B affinity, was decorated through a tertiary protonatable basic head to improve AChE binding affinity and a hydroxymethyl-masking nitrate group eligible for NO release.

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Discovering new casein kinase 1d inhibitors with innovative MD-integrated virtual screening

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The value of including protein flexibility in structure-based drug design and, in particular, in structure-based virtual screening is widely documented and recognized. Molecular Dynamics represents one of the most powerful tools to investigate and simulate protein dynamics, yet the inclusion of MD-derived information is still far from trivial. The huge amount of information in terms of conformations generated by MD has to be filtered to reduce noise and redundancy. In SBVS this generally corresponds to a significant minimal set of conformations to be used in *in silico* screening experiments.

We developed an integrated approach for enhancing accuracy, efficacy, and for conformation selection in VS campaigns, by combining in a pipeline MD, Clustering and the Linear Discriminant Analysis implemented in FLAP (1,2). MD trajectories were clustered according to the Molecular Interaction Fields variation, in order to catch the most representative binding site images, then the LDA chose the best performing conformations, for identifying active ligands among thousands of decoys, thus combining an unsupervised (clustering) with a supervised pre-filtering (LDA). Retrospective analyses on different pharmacological relevant cases recognized the MD-FLAP approach to be a valuable tool for improving VS performances, and confirmed that ensemble receptor protocols outperform single rigid receptor ones (3).

On the basis of these promising results we applied the same procedure on a real case, looking for new possible scaffolds able to target casein kinase 1d. CK1 kinases participate to various cellular processes as DNA repair, cell cycle progression, differentiation and apoptosis, and their deregulation contributes to the pathogenesis of a number of diseases like cancer, neurodegenerative diseases and inflammatory disorders (4). By applying the aforementioned pipeline we obtained a VS model able to separate known actives from inactives on an in-house Pfizer library of about 17000 kinase inhibitors, with a global AUC of 0.9 and a partial ROC enrichment at 0.5% of 0.18, with respect to the 0.77 and 0.036 obtained with a single structure approach. The model was then used in a real VS campaign, screening the internal Pfizer database. The best performing 1000 molecules were filtered according to their structural similarity with known CK1d inhibitors present in the CHEMBL database, looking for new scaffolds. Two new structures were identified and different derivatives analyzed. The best binder showed an IC_{50} of 134 nM. The results supported once more the potential of the integrated MD-FLAP approach in real screening campaigns and the importance of including receptor flexibility for the detection of new ligand scaffolds.

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Isoxazol-5(2H)-one: a new scaffold for potent human neutrophil elastase (HNE) inhibitors

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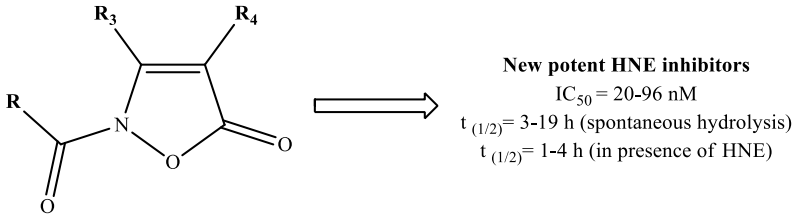
Human neutrophil elastase (HNE) is a serine protease belonging to the chymotrypsin family. It is stored in the azurophil granules of polymorphonuclear neutrophils (PMNs), where it participates in non-oxidative intracellular and extracellular pathogen destruction. HNE plays an important role in many processes, such as blood coagulation, apoptosis and inflammation and exhibits proteolytic activity against a variety of extracellular matrix proteins, like elastin, fibronectin, collagen, proteoglycans and laminin (1). In physiological conditions, the action of HNE is regulated by its endogenous inhibitors (α1-PI, α-2 macroglobulin, SLPI and elafin) but if the balance between proteases and anti-proteases disappears, the excess of HNE activity can cause tissue damage (2). Among the respiratory system pathologies associated with increased HNE are COPD (3), CF (4), ALI and ARDS, but also for rheumatoid arthritis, cancer (5) and neuropathic pain (6) an involvement of HNE was demonstrated. Our interest in the design and synthesis of new non-peptide HNE inhibitors let to the discovery of a potent class of HNE inhibitors with a N-benzoylindazole scaffold (7,8), with IC_{50} values in the low nanomolar range. These compounds are competitive and pseudo-irreversible HNE inhibitors with good selectivity for HNE versus other serine protease and an appreciable chemical stability in aqueous buffer. One of these compound has been also tested in vivo in painful rat models of rheumatoid arthritis (9), osteoarthritis and neuropathic pain. We investigated other scaffold such as cinnoline (10), indole (11) and 7-azaindole and now we have shifted our attention in the design and synthesis of monocyclic nucleous such as isoxazol-5(*2H*)-one which demonstrated to be a suitable scaffold for HNE inhibitors.



R= m-CH_3-Ph, cC_3H_5,
R_3= CH_3, CH_2CH_3, $CH(CH_3)_3$, Ph
R_4= H, CH_3, CH_2CH_3, Ph

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Modelling of Glucuronidation Reactions in the MetaQSAR Database: Successful Strategies to Handle Unbalanced Data in Metabolism Prediction

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Drug metabolism consists of a variety of transformations directly affecting the structure and reactivity of bioactive compounds, and it has a crucial impact on both the efficacy and the safety of drugs. The ability to anticipate such biotransformations is one of the major challenges along the road to producing lead compounds, and computational approaches play a central role in that effort.

Among the classes of metabolic reactions, glucuronidations are unanimously considered the most important reaction type for phase II metabolism, both in qualitative and in quantitative terms (1); however, despite their important contribution, they have been rarely investigated by computational methods. Attempting to make a step towards filling this gap, we are focusing our research on the UDP-glucuronosyltransferase enzymes (UGT) and have developed new integrated predictive models of their activity, exploiting both ligand- and structure-based strategies.

The source of data for our studies is the MetaQSAR metabolic database (2), internally developed and critically collected, which represents a crucial advance over the previous state of the art thanks to the high level of data curation, and provides a reliable data source for model building (3). As expected when dealing with the specific prediction of a single metabolic reaction class (local methods), the dataset collected from MetaQSAR unavoidably includes unbalanced data (399 molecules are UGT substrates and 1421 molecules are not UGT substrates), and this can affect the predictive power of models.

In order to handle this common issue, we present here two different strategies as applied to the prediction of the glucuronidation reactions. The first is based on a machine learning binary classification model, implemented as in the proteochemometric technique, for which we fruitfully exploited the random under-sampling procedure, affording a balanced accuracy of 0.80 (4). The second strategy involves a virtual screening method based on the 3D-structure for the human UGT2B7 isoform (5), recently optimized in our laboratory. This method affords outstanding results, as assessed by enrichment factor analyses (e.g. 100% of substrates ranked in the top 1% and 80% ranked in the top 5%)

Although based on completely different approaches, both models provide very encouraging results and prove successful in addressing the critical issues deriving from unbalanced datasets, which typically challenge metabolic predictive algorithms.

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Comunicazioni Poster

Identification of new KDM4 inhibitors through a HTS and hit refinement strategy

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JHDMs (JmjC-domain-containing histone demethylases) are the largest class of demethylase enzymes, contain a Jumonji C (JmjC) domain and catalyze lysine demethylation of histones through an oxidative reaction that requires Fe(II) ion and α-ketoglutarate (αkG) as cofactors. The misregulation of these enzymes, in particular JMJD2 subfamily, has being significantly implicated in cancer initiation and progression.(1) Potent and specific inhibitors of these enzymes have not been identified yet. Moreover, most of the reported ones show a good affinity to many other Fe(II)/αkG dependent oxygenases, are non-specific for the different isoforms or are affected by undesirable characteristics.(2) By means of an high throughput screening (HTS) campaign, we selected a pool of interesting hit compounds and then, to refine the results, filtered out poor quality scaffolds not suitable for future optimization. The use of a multiple combined approach of different in vitro techniques led us to select EML586 as scaffold for further derivatization. From a series of EML586 analogues we were able to derive a pharmacophore hypothesis and structure-activity relationships (hit-to-lead), and to select 3-hydroxy-2,3-dihydroquinazolinone moiety as starting point for the development of novel optimized derivatives. The substitution of quinoxaline ring with more aliphatic portions gave derivatives such as EML678 and EML684, which demonstrate a better activity against hKDM4A compared to the starting hit compound (Figure 1). Furthermore, they induced a marked reduction in methylation of lysines H3K9 and H3K27 in a cell-based assay together with an arrest in the S-phase of cell cycle.

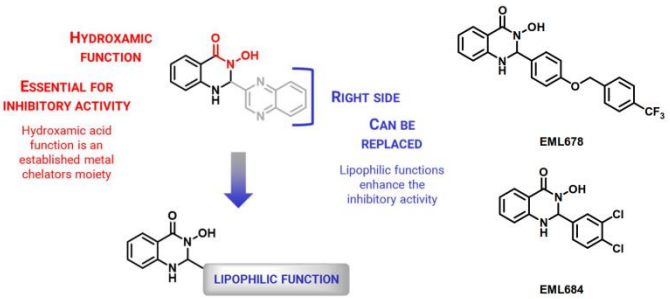

Figure 1. General scheme of our hit derivatization.

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Triazolo quinolone derivatives: a new frontier for the treatment of multi-drug resistant Mycobacterium Tuberculosis strains.

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Tuberculosis (TB) is one of the most common infectious diseases worldwide, with about one-third of world population infected with Mycobacterium tuberculosis (M.Tb.). More frightening is the recent emergence of multi-drug and extensively drug resistant M.Tb. strains (MDR-M.Tb. and XDR-M.Tb., respectively). In the setting of drug resistance or intolerance to first-line agents (e.g. isoniazid, rifampin…), second-line agents may be used. Indeed, fluoroquinolones have been classified as second-line antituberculous drugs since they are active on isolated M. Tb. expressing resistance to both isoniazid and rifampin. Recently we demonstrated that [1,2,3]triazolo[4,5-h]quinolones (TQs) were endowed with a good anti-mycobacterial activity, paired to absence of cytotoxicity (CC_{50} > 100 μg/mL against MT-4 cells). Some of them stood out for their potency against H37Rv and H37Ra and further clinical isolates of MDR-TB/XDR-TB strains (1,4).

Here we present the preliminary development of an interdisciplinary project (5) with the aim to improve knowledge concerning triazolo quinolone derivative scaffold structure-activity relationship (SAR), to identify a pharmacophoric map and enhance the biological activity. New triazolo quinolone derivatives bearing fluorine substitution on the classical quinolone moiety were designed and synthesized to obtain compounds able to inhibit replication in H37Rv and clinically isolated M.Tb. strains bearing different resistance patterns.

All tested derivatives resulted able to inhibit replication in M. Tb. wild type and resistant strains, and no activity resulted when tested on bacterial and fungal strains. The selectivity of action demonstrated by these compounds was investigated through the analysis of their biological target, the M. Tb. DNA-gyrase (wild type and mutated form), which binding site would diverge from classical quinolones.

All data collected, indicating for the compounds a new action mechanism compared to classical quinolones, will be used to create an innovative treatment plan able to reduce possible pharmacological resistances.

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Developing new antimicrobial weapons by combination of Temporin-L with cyclodextrins

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Concern over antibiotic resistance is growing, and new classes of antibiotics, particularly against Gram-negative bacteria, are needed (1). In fact, a lack of new antibiotics for the treatment of Gram-negative infections combined with emerging multi-drug resistance issues demands for the development of new antimicrobial strategies. With an understanding of the pivotal role that cationic host defense (antimicrobial) peptides play in preventing infections by microbial pathogens in many organisms, it has been proposed that these peptides might form the foundation for a new class of clinically useful antimicrobials (2). The therapeutic application of antimicrobial peptides (AMPs) is accompanied by challenges now being resolved owing to an increased understanding of how peptide structure influences mechanism of action. With the aim of overcoming some of the main drawbacks preventing the widespread clinical use of this class of antibacterial therapeutics, i.e. toxicity and unfavorable pharmacokinetics profile, we are designing new formulations combining AMPs with different types of cyclodextrins (CDs) for modulating their hydrophobicity, amphipathicity and degree of α-helicity. Those variations could reduce peptide toxicity as evaluated by measuring their effect on mammalian cell lines. At the same time, peptides-CDs adducts could be more resistant to enzymatic degradation.

We started this project considering the peptide Temporin L (TL), an AMP belonging to the family of temporins. Among AMPs of natural origin, the amphibian temporins represent one of the largest families (more than 100 members) and are among the smallest-sized AMPs (10–14 amino acids) found in nature to date (3). Generally speaking, temporins are known to be active particularly against Gram-positive bacteria. TL is the only exception as it is strongly active also against Gram-negative bacteria and yeast strains, while being strongly hemolytic against human erythrocytes (4).

Here, TL-CDs adducts are evaluated for their antimicrobial activity, toxicity, stability and conformational properties.

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Design, synthesis and in vitro evaluation of bivalent chemical probes for bromo and extra-terminal domain (BET) proteins

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Bromodomains (BRDs) are epigenetic readers that specifically recognize the acetyl-lysine residues of histones. The role in chromatin remodeling and transcriptional regulation correlate these proteins to several disease states such cancer, inflammation, and viral infection, making them an excellent therapeutic target (1). The most studied and druggable family of BRD-containing proteins is the bromo and extra C-terminal domain (BET), whose members (BRD2, BRD3, BRD4, and BRDT) contain two highly homologous bromodomains: BD1 and BD2. Several reports have suggested that these domains have different functions and their selective inhibition could be beneficial in treating diseases or mitigating unwanted effects (2, 3). To date, despite the extensive efforts, there is still a lack of powerful and selective inhibitors of bromodomain proteins, mainly due to the high homology not only between BET proteins but also between BD1 and BD2 domains. Here we describe the design, synthesis and preliminary biochemical evaluation of a new class of bivalent chemical probes of BET proteins (Figure 1). Using different spacers, we linked two different scaffolds: the RVX-208, a selective inhibitor of BD2 domain and a triazolobenzotriazepine-based compound, an inhibitor of BD1 domain. These compounds, simultaneously binding either BD1 or BD2 domains, will help clarify the differences between BD1 and BD2, allowing to get additional details on how these portions recognize the acetylated lysine residues of histones and other proteins.

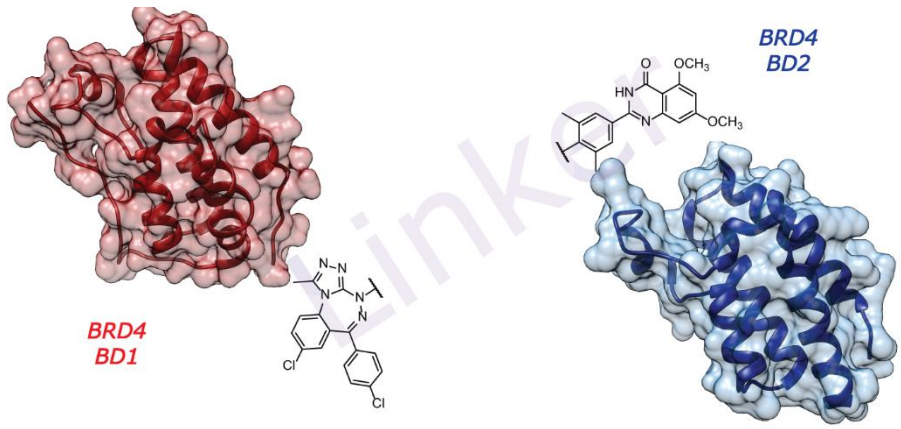

Figure 1 Development of selective and powerful class of BET chemical probes

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Flavonol-like compounds identification as antileishmania agents: chemistry, biology and target studies.

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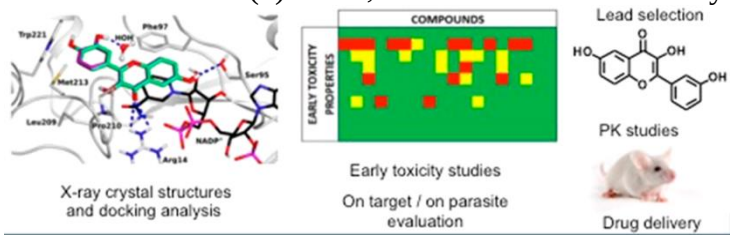
Protozoan parasites of the Trypanosomatidae family are the etiological agents of several significant neglected tropical diseases including human African trypanosomiasis (HAT) Chagas' disease, and leishmaniasis, which collectively aff ect nearly 10 million people worldwide. *Leishmania spp*. infect macrophages and cause a wide spectrum of symptoms ranging from cutaneous lesions to potentially fatal visceral infections (1). Current drugs in therapy show limited efficacy and drug resistance effects, therefore new drugs are urgently needed. A phenotypic approach was applied as a useful tool for drug discovery with the advantage of identifying compounds, which are active against the whole cell. Among a library of natural products, flavonols such as fisetin and quercetin turned out to be potent antiparasitic compounds. Recently, we reported the antiparasitic activity of a library of classical flavonols (2). Thus, the chromen-4-one moiety was confirmed a promising scaffold for the development of antiparasitic compounds. In the present work, we identified a serie of flavonol-like compounds and studied the biological profile against *Leishmania spp*. and targets. Compound CB80 showed an antileishmanial activity comparable to that of miltefosine (EC_{50} vs *L. infantum* H80 = 1.9 μM, Milte = 3.2 μM). We have then evaluated the compound early toxicity profile, the most of the compounds showed low toxicity towards 5 cytochrome P450 (CYPX), human ERG channel and A549 human cells. The best compound, CB80, was selected for pharmacokinetic studies. Snapshot PK studies were performed CB80 showed low stability, therefore cyclodestrin were employed to improve the compound stability. CB80 was tested in mice and hamsters. No animal toxicity was observed, however poor pharmacokinetic and short half-life suggested the need for improving the synthesis of optimized compounds. Drug resistance studies were performed through a genomic approach on sensible and miltefosine resistant *Leishmania* parasite. The drug resistance profile was different from the one observed with miltefosine. This suggests that the compound can be active on miltefosine resistant strains. Target identification studies using differential Mass Spectrometry approaches combined with gel-filtration electrophoresis studies were performed. A comparsion was performed between the proteome of cell treated with CB80 with respect to the untreated one. A protein set of differentially expressed proteins was identified and the results were compared with those obtained through genomic studies. From tthe genomic and proteomic studies we are able to identify those proteins that are relevant for CB80 targeting.



The project was developed within the NMTrypI FP7 European project.

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New inhibitors of Dengue and Zika Virus Protease

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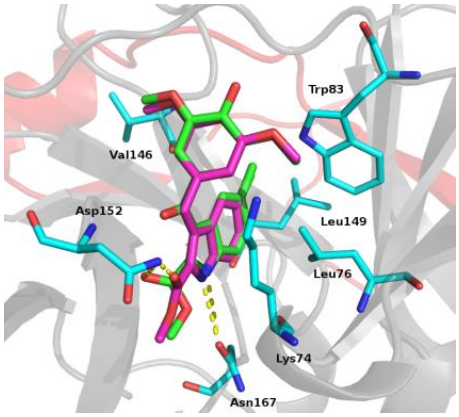
Infections with flaviviruses, such as dengue, and the recently re-emerging Zika virus, are an increasing and probably lasting global risk.
Dengue virus (DENV) is the causative agent of dengue fever, a *Aedes* mosquito-transmitted tropical illness characterized by high fever, severe headache, pain and rash. Currently no licensed vaccines or effective drugs are available, and vector control efforts have not successfully stopped the spread of the infection. There is an unmet need for effective drugs in the treatment of DENV infection. Zika virus (ZIKV) is a mosquito borne pathogen, belongs currently known for causing large epidemics in Brazil. The recent outbreak of ZIKV demands an enhanced surveillance and a need to develop novel drugs against ZIKV.
In search for new DENV protease inhibitors we carried out virtual screening (VS) studies on the NS2B/NS3 protease. Thanks to our virtual screening we were able to identify some derivatives showing promising inhibitory activity against the DENV protease at one digit micromolar concentration (Chart 1A).[1] Due to the close relationship between ZIKV and DENV, we tested if highly active anti-DENV compounds could be used as an advanced starting point for the discovery of ZIKV NS2B/NS3 protease inhibitors.
In particular, compound **1** (Chart 1B) proved to be a valuable inhibitor against ZIKV protease and paved the way for design on new more potent dual inhibitors.

Chart 1. New DENV and ZIKV protease inhibitor

A



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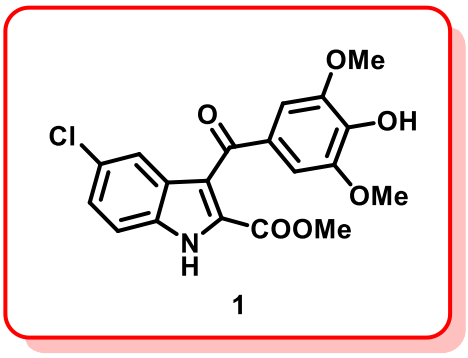

References: 1 Pelliccia, S. *et al*. Inhibition of Dengue Virus Replication by Novel Inhibitors of RNA-dependent RNA Polymerase and Protease Activities. *JEIMC, submitted.*

Biomolecular and biophysical approaches for the identification of chemical probes for the PHF20 Tudor2 methyllysine reader domain

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Among epigenetic enzymes, writer and eraser proteins have been the main focus of therapeutic development but over the past few years a relatively underexplored group of proteins, the readers, have emerged as promising targets operating at the interface of translating histone marks. While their importance in several biological processes is evident, there is a strong need to identify new modulators to be used as chemical probes to better understand the role of these proteins in physiological and pathological states.

Plant homeodomain finger protein 20 (PHF20) is a multidomain protein mainly involved in the activation of p53 and in the prevention of its ubiquitylation (1). Furthermore, it uses the second Tudor domain to read dimethyl lysine residues and it plays a role in the cross-talk between lysine methylation and histone acetylation (2).

With the aim to identify chemical probes for different methyllysine reader domains (3), we synthesized a library of compounds that were used to challenge a microarray of reader proteins. This approach allowed us to identify very promising hits (4). We herein describe the development of a robust combined biochemical and biophysical screening platform for the validation of the identified hits for the Tudor domain 2 of PHF20 and their full characterization. In order to deeply characterize the key elements for the interaction of the modulators with the target protein, we used different protein sequences and we evaluated the influence of the presence of different tags. This combined approach represents a powerful method for measuring readers activity and it allowed us to identify new chemical probes, very useful for the study of the activity of this reader and its implications in physiological and/or pathological processes.

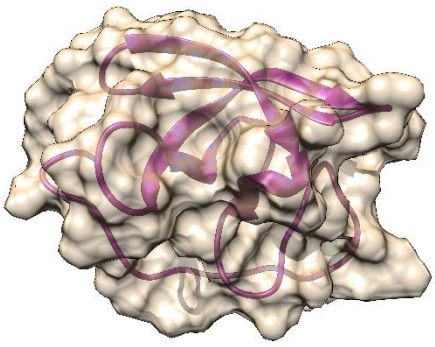

Figure 1. *Plant homeodomain finger protein 20 (PHF20)*

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Quinoxaline derivatives as new leads against Picornavirus

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Picornaviruses are viral agents which cause a wider range of illnesses than most other, if not all, virus families. The Picornaviridae family comprises five genera, namely Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, and Aphthovirus. Infection with various Picornaviruses may be asymptomatic or may cause from mild illnesses (the common cold, febrile rash illnesses, conjunctivitis…) to serious conditions affecting the central nervous system (encephalitis), heart (myocarditis), skeletal muscles (myositis), and liver (hepatitis). (1)

Human Enteroviruses (EVs) are relevant pathogens circulating commonly in the environment, with a seasonal peak during early fall. Coxsackievirus belong to this genus and are noted to cause systemic disease after ingestion and replication in the gastrointestinal tract (2). Actually, no specific antiviral agent is approved by the US Food and Drug Administration for the treatment of Enterovirus infections.

In this poster, we report the synthesis and the *in vitro* and *in silico* antiviral activity of a series of new quinoxaline derivatives. All compounds were tested for cytotoxicity and biological activity against a wide panel of representative ssRNA, dsRNA and dsDNA viruses. From all compounds, three quinoxaline derivatives stood out for their very potent and selective activity against Coxsackievirus B5, with EC_{50} values in the sub-micromolar range (0.3 - 0.06 μM). The most active, selective and not cytotoxic compound, 2-[6-(2,3-dimethoxyquinoxalin-6-ylmethylthio)pyridine-3- carboxamido]-L-glutamic acid] (**7a**) was widely evaluated using a combination of experimental techniques (i.e., virucidal activity, time of drug addiction, and adsorption assays) and preliminary data are here reported. These data were finally used to hypothesize the antiviral mechanism of action, and since activity of **7a** towards CVB-5 is only 10 time higher (EC_{50} = 0.09 μM) that the one measured for the same cell line treated with pleconaril (EC50 = 0.005 μM), we hypothesized that these two compounds might exert a similar mechanism of action as viral capsid protein binders. To confute *mechanicistically* the hypothesis, molecular modelling studies were further performed.

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Pyrrolyl non-dka derivatives as novel inhibitors of hiv-1 reverse transcriptase-associated ribonuclease h function

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The AIDS is a complex of pathological manifestations characterized by progressive degeneration of the immune system caused by the HIV virus. An essential enzyme for the retroviral life cycle is reverse transcriptase (RT), an heterodimeric enzyme with two associated activities: the DNA polymerase activity and the ribonuclease H (RNase H) activity that selectively degrades the RNA strand of the hybrid RNA/DNA formed during the synthesis of the minus (-) strand DNA that uses (+) RNA as a template.(1)

Despite such a large armamentarium, both acute and chronic toxicities limit the prolonged use of several antiretroviral agents, and this is even more a concern because of the life-long character of the therapy. In addition, the selection of drug-resistant strains and the spreading of such strains in newly infected patients is also an increasing concern, underscoring the pressing demand of novel anti-HIV agents, with a better therapeutic index and a very broad spectrum of activity against the mutants, possibly targeting viral functions not yet explored.(2)

In such a scenario, an attractive target turns out to be the RNase H function of HIV-1 reverse transcriptase (RT), which has been little explored although it could be potentially vulnerable to a specific inhibition. (3,4,5,6)

Although RT is a multifunctional enzyme, all RT inhibitors currently approved for the treatment of HIV infection target only the RT-associated polymerase function, while none of them block the RT RNase H activity. Nevertheless, several studies have demonstrated that the abolition of the HIV-1 RNase H function stops the virus replication, proving to be, therefore, a validated and attractive target for the development of new anti-retroviral agents, in order to enhance the anti-HIV-1 drug armamentarium effectiveness. Despite this, it has been little explored and it needs to be further developed through the support of new HIV/AIDS drug discovery programs, in order to identify more efficient anti-HIV drugs that could be used for therapy.(7,8)

To date, only few compounds have been described to inhibit the HIV-1 RNase H function. Among them, aryldiketo acid derivatives proven to inhibit both integrase enzyme and RNase H function of the RT.(9,10) Pursuing our studies on pyrrolyl DKA derivatives as dual inhibitors of IN and RNase H we developed non DKA scaffold and found a new class of compounds that selectively inhibited the RNase H. The data coming from the biological assays will be shown and discussed.

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StOASS inhibitors as putative new antibacterial agents

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Multidrug resistant (MDR) bacteria are challenging the efficacy of the available antibiotics to treat common infections and minor injuries both in the community and hospitals. Statistical data have estimated that around 23,000 - 25,000 people die each year as a result of a superbug infection.(1,2)
De novo cysteine biosynthetic machinery, which is exclusive in prokaryotes, has been associated with the growth, survival and pathogenicity of several bacterial species. (3,4) Therefore, inhibition of the cysteine synthase complex, the result of the association between O-acetylserine sulfhydrylase (OASS) and serine acetyltransferase (SAT) enzymes, may provide a new therapeutically relevant target against MDR strains.
To obtain the first inhibitors of OASS, several peptides were assayed on the recombinant enzyme from *Salmonella typhimurium*. (5) However since peptides present major drawbacks as chemotherapeutical tools a campaign aimed to obtain the first small molecule inhibitors of OASS was started.(6,7) Compounds with low nanomolar activity were obtained and then assayed on bacteria. Nevertheless, despite the high inhibitory activity the most promising compound wasn't able to interfere with bacterial growth. Further investigation presented permeability as the main cause of the lack of antibacterial activity. Therefore, starting from the structure of the most promising compound and with the aim of improving its pharmacokinetic properties, we herein present the synthesis and biochemical evaluation of a new series of StOASS inhibitors.

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Modulation of cell differentiation through HDAC inhibitors

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The induction of pluripotency to produce embryonic-like stem cells as well as the modulation of cell differentiation pathways through small molecules are major topics in stem cell research. Reprogramming of somatic cells has been attempted using different methods: somatic cell nuclear transfer, transduction of pluripotent genes into somatic cells, somatic cell fusion with pluripotent cells, and pluripotent cell extract mediated de-differentiation. The reprogramming processes of somatic cells are however still unrewarding and counteracted by the use of viral vectors eventually leading to permanent host genomic integration of foreign genetic materials. Recently, small molecules able to modulate specific targets in receptor signaling and epigenetic machinery have been used to improve the reprogramming process and/or replace some transcriptional factors, thus partially or totally avoiding the host genome involvement (1). In this context, histone deacetylase inhibitors (HDACIs), such as valproic acid (VPA), thricostatin A (TSA), and suberoylanilide hydroxamic acid (SAHA), induce the hyperacetylation of histones thus modifying chromatin moiety and affecting gene expression (2). Although they are mainly used in anticancer therapy, these compounds have been successfully tested as reprogramming agents.
To evaluate the ability of HDAC inhibitors (HDACi) in reprogramming cell differentiative potential (3), we have designed and synthesized new hydroxamic acids. The compounds have been tested on primary human fibroblasts cultured *under standard conditions (control samples) or induced* into *adipogenesis, myogenesis and neurogenesis with known differentiative inducers (treated samples). The cellular response has been evaluated by immunofluorescence (vimentin, leptin),* Real-time PCR analysis *(RT-PCR) (*myogenic differentiation factor *1, myogenin, tropomyosin*, brain-derived neurotrophic factor, nerve growth factor*, tubulin β3, synaptophysin SYP) and western blot (*matrix metalloproteinases *2, 9, 13) of specific cell lineage markers. In parallel, the morphology and functionality of* exosomes and microvesicles (4) from HDACi-treated samples and controls have been *characterized by scanning electron microscopy and RT-PCR (*pluripotent transcription factor mRNAs, growth factors, cytokines, immune regulators).

The present work has been carried out with the financial support of University of Padova (CPDA120753/12)

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Drug design and synthesis of new indolylarylsulfones as HIV-1 non-nucleoside reverse transcriptase inhibitors

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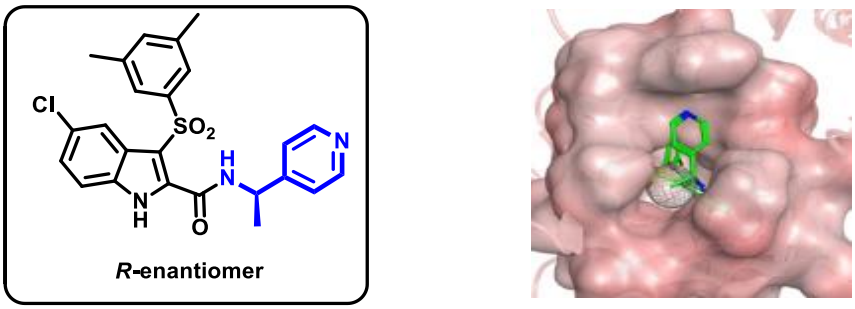
HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key drugs of highly active antiretroviral therapy (HAART) in the clinical management of AIDS/HIV-1 infection. Our recent studies showed that indolylarylsulfones (IASs) bearing a cyclic moiety at the 2-carboxamide nitrogen linked through a short spacer group were endowed with potent antiretroviral activity.[1,2]
Based on the results previously obtained, we aimed to expand the SAR studies by the introduction of new aryl or heteroaryl portions to the indole nucleus.
Interestingly, for the first time IASs endowed with asymmetric centre have shown significant differences in term of antiretroviral potency. In particular, the *R*-enantiomer proved to be exceptionally potent and uniformly superior to the *S*-enantiomer against the whole viral panel. Docking studies showed that the methyl group of the *R*-enantiomer (Figure 1) pointed toward the cleft created by the K103N mutation, differently from the corresponding group of (S) counterpart. By calculating the solvent accessible surface, we observed that the exposed area of the RT in complex with *S*-enantiomer was larger than the area of the (R) complex.[3]

Figure 1.



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Thiophene-3-carboxamides and triazolopyrimidine-2-carboxamides as precious scaffolds to disrupt influenza polymerase PA-PB1 subunits heterodimerization.

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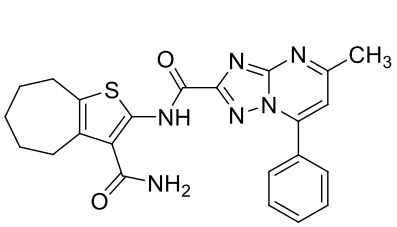
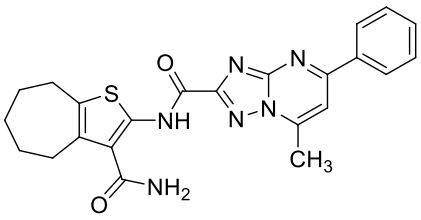
The limited therapeutic options against the influenza virus (flu) along with drug resistance issue make imperative the search for next-generation agents. In this context, heterotrimeric viral RNA-dependent-RNA-polymerase (RdRp) is a valuable target for a challenging but strategic protein-protein interaction inhibition approach. Since 2012, the inhibition of the RdRp PA-PB1 subunits interface has become an active field of research, following the publication of PA-PB1 crystal structures (1).
Our group has identified many of the PA-PB1 complex formation inhibitors reported to date, thanks to an initial SBVS that led to identify five hit compounds, followed by their optimization (2-4). The most enthusiastic result was achieved with the identification of two hybrid molecules (compounds **1** and **2**) obtained by merging the triazolopyrimidine and cycloheptathiophene scaffolds characterizing two of the hit compounds. Indeed, compound **1** emerged as the most potent PA-PB1 small molecule inhibitor developed thus far (4).



1



2

To further optimize compounds **1** and **2**, two efficient and region-selective one-pot synthesis were developed to prepare 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino[1,2,4]triazolo[1,5-a]pyrimidine derivatives, as key intermediates in the synthesis of an enlarged series of hybrid analogues.
In this work, their design, synthesis, and biological evaluation will be presented.

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Identification of new inhibitors of PRMTs by a multi-substrate-adduct approach

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The methylation of arginine residues is a prevalent posttranslational modification found in both nuclear and cytoplasmic proteins, which is involved in a number of different cellular processes, including transcriptional regulation, RNA metabolism, and DNA damage repair. Enzymes of the protein arginine *N*-methyltransferase (PRMTs) family catalyze the transfer of a methyl group from the donor *S*-adenosyl-*l*-methionine (SAM or AdoMet) to the guanidinium side chain of arginine residues in the target protein. Despite extensive research aimed at better understand the role of PRMTs in physiological and pathological pathways, there have been only a few publications to date describing small-molecule chemical modulators of the PRMTs. A few years ago, starting from **AMI-1** (the first selective inhibitor of PRMTs) (1) we identified **EML108**, which was characterized by an improved selectivity profile among methyltransferases and a good cellular activity (2). Moreover, docking studies clearly showed that **EML108** bind SAM and arginine pocket without fully occupying them. Starting from this evidence, we herein report the design and the synthesis of new PRMTs inhibitors based on the naphthalene scaffold of **EML108**. Firstly, we prepared some derivatives bearing a guanidine moiety connected to the naphthalene scaffold *via* a variable linker. After optimization, we further functionalized this scaffold with an adenosine moiety (Figure 1). This multi-substrate-adduct approach lead to the identification of new sub-micromolar inhibitors of PRMTs.

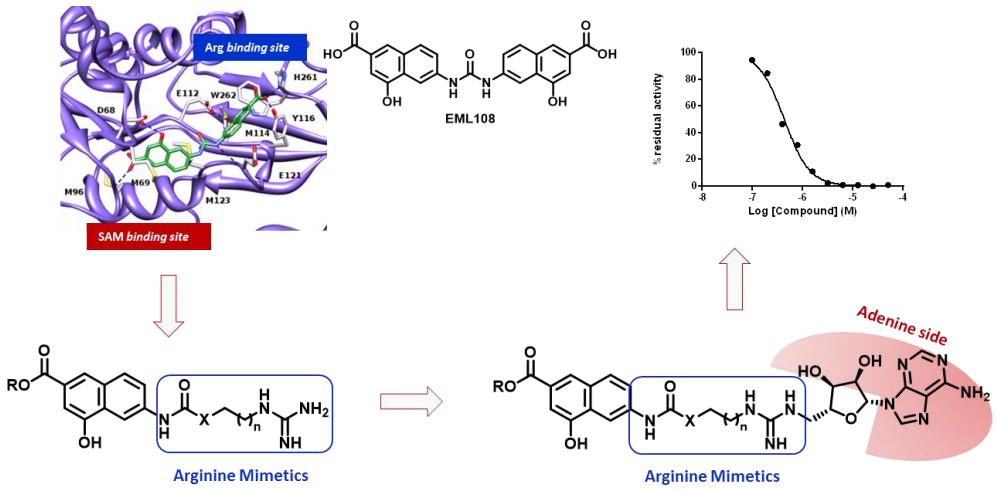

Figure 1: Multi-substrate-adduct approach to the discovery of new inhibitors of PRMTs

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Design and synthesis of a new anti-Chitinase compound

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In the last ten years, we identified and developed a new therapeutic class of antifungal agents, the macrocyclic amidinoureas (1). These compounds act on various *Candida* species, including clinical isolates resistant to currently available antifungal drugs (2). The mode of action of these molecules is still unknown. Therefore, we developed an *in-silico* target fishing procedure to identify a possible target for this class of compounds. Chitinase enzyme emerged as possible target. To confirm this hypothesis a novel macrocyclic derivative, compound **2**, has been synthesized (Fig. 1). This compound has been specifically designed to increase the inhibition of the Chitinase; to achieve this, we thought to merge **1** with Argifin (a natural compound known to be a good Chitinase inhibitor) (3) that assumes a similar pose and shape to **1** when docked against Chitinase. The aim of this step is to test if an increase in the enzymatic activity is reflected in the antifungal activity.

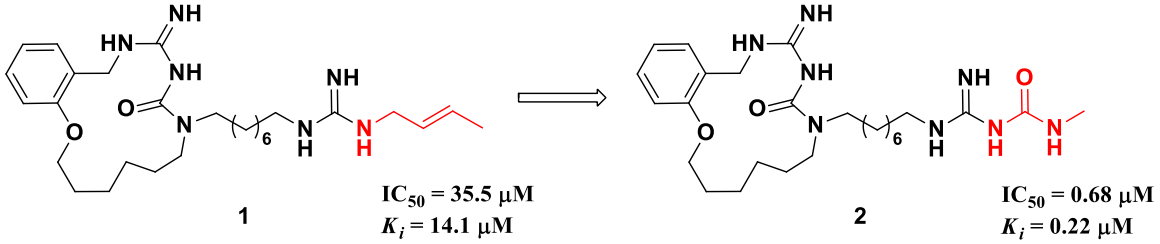

Figure 1: Compound **1** and **2**

The optimized derivative was tested against *T. viride* Chitinase and it exhibited a potent enzymatic inhibition, almost 50-fold lower than compound **1**. This confirmed the robustness of our computational model. Its antifungal activity, though, is lower than the parental compound. This could be due to the poor membrane penetration of **2**, due to the transformation of the positive-charged terminal guanidine (pKa ≈ 12) to a neutral amidinourea (pKa ≈ 6). It is also possible that chitinase represents only one of the targets for this class of compounds, and these modifications reduced the affinity for the other targets. More investigations on this aspect need to be done.

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From a serendipitous discovery to new alkyl-guanidine oligomers as perspective antibacterial agents

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The rapid emergence of resistant bacteria is occurring worldwide and nowadays it is one of the major threat to human health, leading to the loss of the efficacy in the treatment of infectious diseases. (1) Thus, new chemical classes with innovative mode of action are required to prevent this crisis. (2) Our research group recently reported the identification of a series of linear guanidine derivatives and their antibacterial properties. (3) A batch of a promising candidate for optimization studies (compound **1**) turned out to be a mixture containing two unknown species and surprisingly it showed a better biological activity than the pure compound (MIC = 64 µg/mL). After this serendipitous discovery, we put efforts into the investigation about the chemical nature of the unknown components of the mixture and by means of MS analysis interfaced with the synthesis we found that the components were oligomeric derivatives of compound **1**.
Eventually, we identified a new family of compounds endowed with broad-spectrum antibacterial activity on both Gram positive and Gram negative strains. Among the synthesized compounds, the symmetric dimeric derivative **2** exhibited the best profile (MIC values ranging from 1 to 8 µg/mL) and it has been highlighted as a perspective lead compound for further studies. (**Figure 1**).

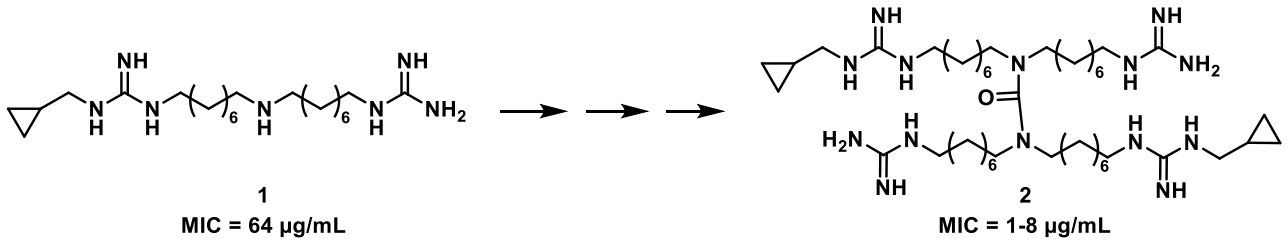

Figure 1. Compound **1** and its symmetric dimeric derivative **2**. MIC values in µg/mL are shown for both compounds.

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Evolution of N-phenyl-5-(2-(phenylamino)thiazol-4-yl)isoxazole-3-carboxamides as valuable antitubercular candidates

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Tuberculosis remains one of the deadliest infectious diseases in the world, and the increased number of multidrug-resistant and extremely drug-resistant bacterial strains is a significant reason of concern.(1,2) This makes the discovery of novel antitubercular agents a cogent priority. We have previously addressed this need by reporting a series of substituted 2-aminothiazoles capable to inhibit the growth of actively replicating, non-replicating persistent, and resistant *Mycobacterium tuberculosis* strains.(3) Clues from the structure–activity relationships lining up the antitubercular activity were used for the rational design of improved analogues. Two compounds, in which the 2-aminothiazole core is linked to an *N*-substituted isoxazole-3-carboxyamide, were found to possess high inhibitory activity toward susceptible and resistant *M. tuberculosis* strains, along with other favorable pharmacological characteristics such as metabolic stability, selectivity, and lack of toxicity toward macrophage cell lines.(4) Based on the structure of these interesting leads, different derivatives were synthesized in order to improve activity, define structure-activity relationships and refine drug-likeness. The preparation of such molecules was based on traditional organic chemistry combined with microwave heating. All of the synthesized compounds were preliminarily evaluated through a MABA assay: some of them were shown to possess very good activity against MTb, in some cases better than the lead compounds, and all showed a lack of toxicity when texted toward VeroCells. These results, since the detailed SAR and drug-like characteristics, encourage pursuing further efforts toward the rational synthesis of new derivatives.

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Lipase-catalyzed synthesis of pinocembrin derivatives as potential antibacterial agents

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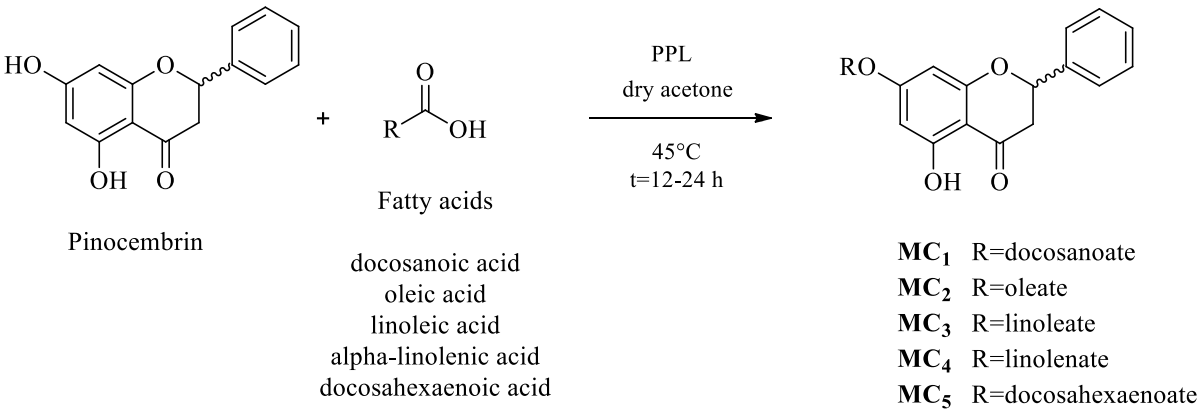
Antibiotics are typically antibacterial drugs that interfere with some structures or processes which are essential to bacterial growth or survival. Antibiotic-treatment failure is generally attributed to resistance. Nowadays antibiotic resistance has spread at an alarming rate. Many resistance mechanisms have been identified, including mutations that decrease the binding of the drug to its target and increase expression of efflux pumps. The effect of such mutations is measured by the minimum inhibitory concentration (MIC), the lowest drug concentration needed to prevent the visible growth of the microorganism (1). Since the abuse and the inappropriate use of antibiotics have caused an increase of this phenomenon, scientific research aims at detecting new antibacterial agents. In this work we describe the synthesis of new molecules derived from a chemical modification of pinocembrin, one of the most abundant natural compound isolated from *Glycyrrhiza glabra* L. leaf. After a classical maceration extraction, GC-MS analysis of the organic layer (n-hexane) have revealed the presence of several fatty acids showing an interesting antibacterial activity. We merged pinocembrin, mainly present in the methanol layer and already reported for its antibacterial activity, with a series of fatty acids by a lipase-catalyzed esterification (2). We chose both saturated and unsaturated fatty acids, with a different length, to highlight differences in antibacterial power according to the chemical structure.



The obtained results have proved that MC_3 is the derivative with the best antibacterial activity.

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Design, synthesis and biological evaluation of novel G9a inhibitors with improved brain permeability from a scaffold hopping approach

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The lysine methyltransferase G9a (also known as EHMT2) catalyses the addition two methyl groups to lysine 9 of histone H3. Due to its central role in epigenetic control, the aberrant activity of this enzyme is associated to several diseases including cancer. In particular, recent evidences revealed G9a involvement in the progression of REST-expressing (repressor element (RE)-1 silencing transcription factor) medulloblastomas. (1) Only a few among the selective inhibitors of G9a reported to date are useful chemical probes for cell-based and animal studies. (2)

Starting from the inhibitor UNC0638, (3) we applied a scaffold hopping approach to develop novel chemical entities endowed with high affinity towards G9a. In particular, we replaced the quinazoline core, common to most of the reported inhibitors, with 1,4-benzodiazepine nucleus, known to be a privileged structure. We chose the 3,4-dihydro-5H-benzo[*e*][1,4]diazepin-5-one scaffold, that can be obtained through an efficient and gram-scale continuous-flow protocol, previously optimized by our group. (4) Moreover, this scaffold could be easily decorated to provide a number of highly functionalized potential ligands (Figure 1). To validate our approach, we designed and synthesized a small library of UNC0638 analogues. The UNC0638 benzodiazepine analogue (EML741) showed a good activity in a peptide-based AlphaLISA, together with a promising membrane permeability profile (PAMPA-BBB).

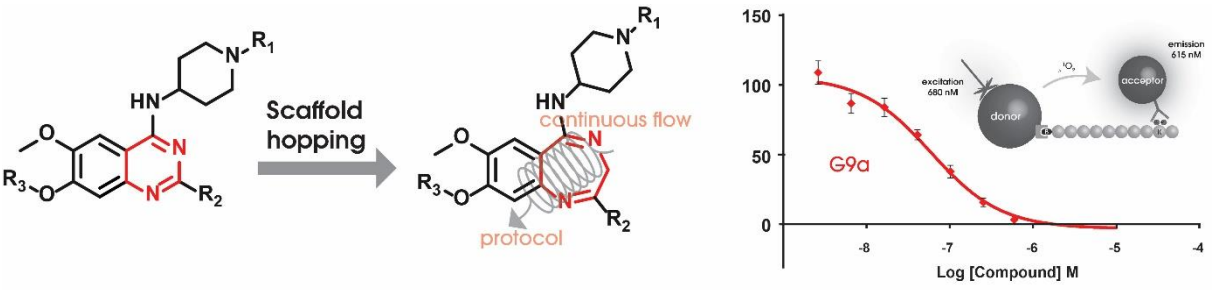

Figure 1. General scheme of our scaffold hopping approach

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Derivatives of 2-amino-6-fluorobenzoic acid as inhibitors of *Mycobacterium tuberculosis* Tryptophan biosynthetic pathway

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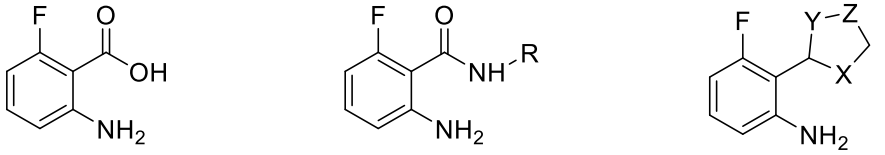
In spite of the enormous efforts that have been made in the hunt for new drugs, tuberculosis (TB) still remains the leading bacterial cause of mortality worldwide, causing an estimated 10.4 million new cases and 1.8 million deaths in 2015 (1). Recent studies have demonstrated that *Mycobacterium tuberculosis* (*Mtb*) survives host CD4-generated stress by production of tryptophan (Trp), thus avoiding starvation and rendering the host immune response ineffective (2). Thus, molecules that can inhibit Trp biosynthetic pathway could synergize with the host immune response to eradicate *Mtb* infection. Moreover, Trp is an essential amminoacid for humans, so anti-Trp synthetic drugs should have limited mammalian toxicity. Therefore, Trp biosynthetic pathway is a valuable target for anti-TB drug development.

A 2-amino-6-fluorobenzoic acid (6-FABA) (Fig. 1) has been recently identified, whose bactericidal activity against *Mtb* was observed only in the absence of Trp, consistent with this compound acting by targeting tryptophan biosynthesis (2).

Herein we present a series of 6-FABA new analogues synthetized for optimizing the antimycobacterial activity and to improve both the drug-like properties and the pharmacokinetic profile of the parent compound (FABAs 1-37, 39-43, Fig. 1). Among the newly synthesized compounds the hydrazides FABA 14, 16, 24, 26, 27, 29-31, 34, 37, 40 and 41 have shown an outstanding antimycobacterial activity.



6-FABA **FABA 1-19, 23-41, 43** **FABA 20, 22, 42**

Figure 1. Chemical structure of 6-FABA and FABAs 1-37, 39-43.

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Identification of novel small-molecule ligands of methyl-lysine binding protein PHF20

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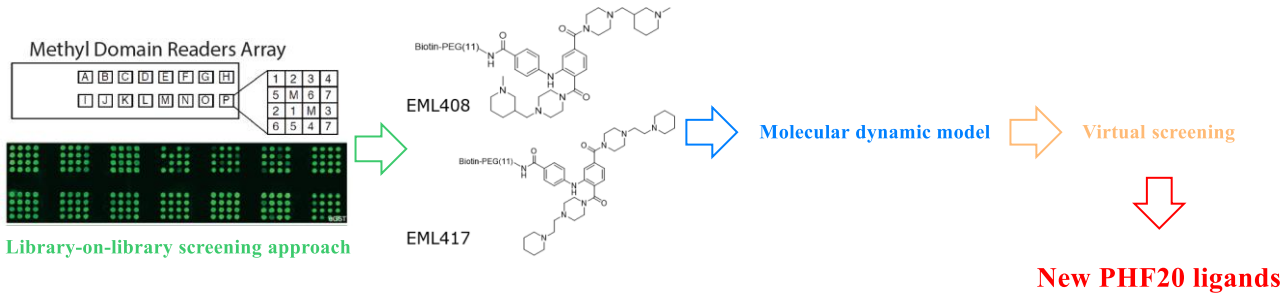
Methylation of histone tails influences overall chromatin structure and the accessibility of DNA segments, thus representing a crucial post-translational modification involved in gene regulation. Recognition of these methyl marks has been attributed to the "Royal Family" of proteins, which includes the Tudor domain subfamily. The ability of these enzymes in binding lysine-methylated protein substrate has been well documented (1, 2). However, much remains to be elucidated with regard to precise mechanisms by which such interactions influence the processes of transcription, translation and RNA splicing.

Among the "readers", the Plant Homeodomain Finger protein 20 (PHF20) is a transcription factor, which was originally identified in glioma patients (3). While little is known about its cognate cellular role, PHF20 is prevalent in hepatocellular tumors of stage I (4) and is also abundantly expressed in both advanced small-cell lung cancer and advanced adenocarcinoma, indicating that PHF20 might be tumor-associated antigen and could play a role in cancer progression.

Starting from a 'library-on-library' screening approach, compounds that selectively bound the Tudor domains of PHF20 were identified (**EML408** and **EML417**, Figure 1). A molecular dynamic model was also used to understand the right length to allow optimal interactions of the new ligands with the two cages of PHF20 dimer.

Prompted by our interest in the discovery of small molecule modulators of epigenetic targets, after structural optimization as well as virtual screening studies, here we report the identification of a series of inhibitors of PHF20, that might represent new opportunities to investigate the role of this protein in chromatin biology and drug discovery.



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Discovery of naphtalimide-based non-ATP competitive GSK-3β inhibitors by an ESI-Q-TOF method

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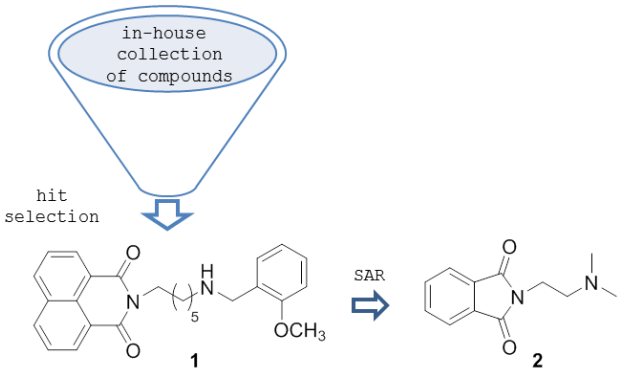
Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine kinase largely expressed in the central nervous system (CNS), which proved to play a significant role in regulating tau phosphorylation under both physiological and pathological conditions, being implicated in the formation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs). In particular, GSK-3β dysregulation is assumed to contribute to the aetiology of chronic conditions such as cancer, and Alzheimer's disease (AD).[1] However, most of the available GSK-3β inhibitors binds to the ATP-binding site which is highly conserved in all the human kinome[2]; therefore, such agents are endowed with low selectivity. Nowadays, only few non-ATP competitive GSK-3β inhibitors are available and, in light of these considerations, the discovery of agents acting through this mechanism of action is highly desirable.

Aim of the present investigation was the design of new low molecular weight non-ATP competitive GSK-3β inhibitors. To reach this goal, a straightforward ESI-QTOF method, enabling fast hit selection and detailed kinetic characterization of GSK-3 inhibitors, was developed.[3] Taking advantage of this new methodology, an in-house collection of compounds was screened towards GSK-3β, leading to the discovery of **1**, a prototype of a new class of hits able to inhibit GSK-3β. Following Structure-Activity Relationships campaign, we discovered compound **2** characterized by a Ki value of 3.49 μM. The kinetic analysis carried out by this new spectrometric method revealed compound **2** as a nonATP-competitive mechanism of action.[4]



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Design, synthesis and biological evaluation of triazolopyrimidinium salts as novel antiproliferative agents

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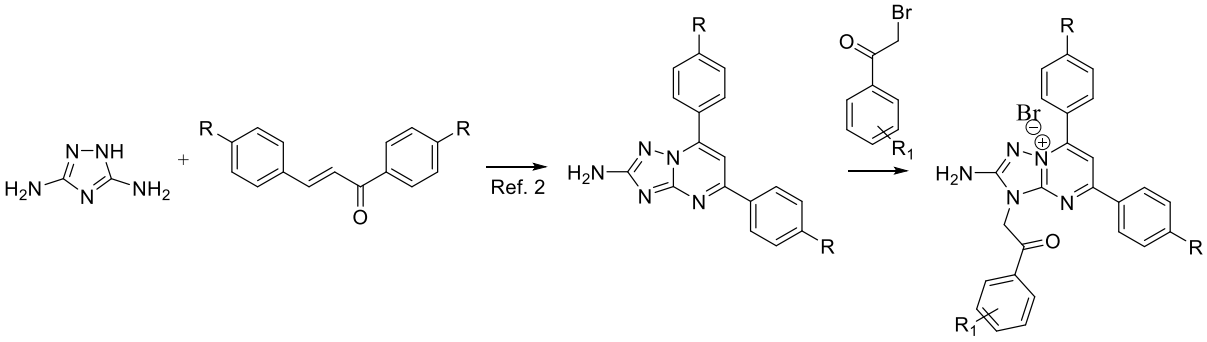
Nowadays, cancer is one of the most common diseases in the world, associated with the highest mortality rate. According to the cancer statistics from National Cancer Institute (NCI), more than a million and a half of new cases of cancer have been estimated in 2017 and almost a third of diagnosed people will die. In general, the lack of evident or specific signs or symptoms makes cancer difficult to detect. Moreover, treatments for this deadly disease, including chemotherapy, radiation therapy and surgery when possible, depend on the type and the stage of cancer and most of times they are not completely successful and effective (1). Taken together, these statistics show how urgent is the discovery of new drugs to treat cancer. With this purpose, we identified the triazolopyrimidine nucleus as scaffold for the design of new antitumor agents. The salts were obtained from the triazolopyrimidine nucleus, firstly synthesized following the procedure described by Desenko et al. (2), by N-alkylation with bromoacetophenone, bearing various substituents on the aromatic ring.



The amino group was also subject of alkylation and acylation to enlarge our library of compounds. A primary screening against several different tumor cell lines showed that the new synthesized salts have a good antiproliferative effect, particularly against pancreatic cancer cells. Preliminary study showed that some derivatives may induce ROS production. Further mechanistic assays are ongoing to establish the pathway by which they cause cell death.

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Discovery and characterization of potent F508del-CFTR correctors

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Cystic fibrosis (CF) is a fatal genetic disease affecting approximately 1 in circa 2500 live births in the Caucasian population. The disease is caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene that result in loss-of-function of the CFTR protein, an ion channel involved in Cl^- and HCO_3^- transport in multiple organs. The most frequent mutation among patients with CF, the deletion of phenylalanine at position 508 (F508del), causes a defective maturation and impaired gating of the CFTR protein. The maturation defect can be treated with compounds known as correctors, whereas the gating defect can be overcome by compounds called potentiators (1). Only one corrector has been approved, in combination with a potentiator, for the treatment of CF patients bearing the F508del-CFTR mutation, i.e. lumacaftor (VX-809), but the therapeutic benefit of the combination is limited. There is therefore the need of new, more effective correctors.

To discover new correctors, the D3's compound collection, containing around 15,000 maximally diverse commercial compounds, was screened in two different cell types, FRT and CFBE41o- stably expressing F508del-CFTR and the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP) (2). Primary hits from the high throughput screening were tested at 6 different concentrations in the same cell types and those showing dose-dependent activity were confirmed in secondary assays. Two confirmed hits, belonging to two different chemical classes, were selected for investigation of the Structure-Activity Relationships (SARs).

The medicinal chemistry work lead to compounds with improved potency and efficacy with respect to the confirmed hits. A set of correctors showed high efficacy and potency in the low nanomolar range when tested in the HS-YFP assays. Further characterization of those compounds in the Trans-Epithelial Electrical Conductance (TEEC) assay, run on F508del-CFTR FRT cells, confirmed their high efficacy and potency. Finally, the most interesting correctors were tested in primary bronchial epithelial cells from CF patients homozygous for the F508del mutation. A number of compounds showed efficacy comparable or superior to that of the VX-809. Most interestingly, a few compounds retained very good efficacy at a concentration as low as 10 nM, a concentration at which no activity was observed for VX-809. The modifications of the confirmed hits to increase their activity were also accompanied by an improvement of their drug-like properties. The data generated on the most promising correctors will be presented and discussed.

This work was supported by the Italian Foundation for Cystic Fibrosis (FFC) as part of the "Task Force for Cystic Fibrosis" project.

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The kinase inhibitor pyrazolyl-urea GeGe3 inhibits angiogenesis and reveals dystrophia myotonica protein kinase (DMPK)1 as a novel angiogenesis target

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Activation of alternative receptor tyrosine kinases by compensatory angiogenic factors was implicated in the failure of targeting VEGF/VEGFR2 signalling in cancer therapy. Targeting MAPK and PI3K signaling pathways, commonly induced by angiogenic factors, may be an alternative approach. In previous studies, we developed several chemical libraries able to block activation of ERK1/2, p38MAPK and AKT in neutrophils stimulated by IL-8 or formyl-methyl-leucyl-phenylalanine (fMLP) peptide and inhibit neutrophil migration (1,2 and references therein cited). More recently we designed and synthesized a large series of pyrazolyl-ureas and imidazo-pyrazole-carboxamides and found them to differently modulate the activity of ERK1/2, p38MAPK and AKT in human umbilical vein endothelial cells (HUVEC) stimulated by VEGF (3). Our library revealed the ethyl 1-(2-hydroxypentyl)-5-(3-(3-(trifluoromethyl)phenyl)ureido)-1*H*-pyrazole-4-carboxylate (named GeGe3, Fig. 1) to be an inhibitor of HUVEC migration. This suggested that GeGe3 may be a potential blocker of angiogenesis.

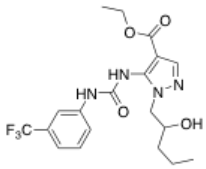

Figure 1. Compound GeGe3 structure

GeGe3 was further analysed in vitro on proliferation of HUVEC and cancer cell lines, and in vivo on physiological angiogenesis in Tg(fli1a:EGFP)y1 zebrafish embryos as well as pathological angiogenesis in Lewis Lung carcinoma LLC1 tumors in C57BL/6 mice. GeGe3 targets were identified by using Pamgene®12 arrays. The candidate kinases were further characterized biochemically and their relevance in angiogenesis was challenged.

Results: GeGe3 blocked ERK1/2 and AKT activation and inhibited the migration and proliferation of HUVEC, but showed no effect on proliferation of human and mouse cancer cell lines in vitro. Accordingly, GeGe3 impaired intersegmental angiogenesis during development of zebrafish embryos. In mice, GeGe3 blocked angiogenesis and tumor growth in transplanted subcutaneous Lewis Lung Carcinomas (LLC1). Screening for GeGe3-targeted kinases revealed Aurora B, Aurora C, NEK10, polo-like kinase (PLK)2, PLK3, DMPK1 and CAMK1 as candidate targets. In-depth examination revealed DMPK1 as a new mediator of angiogenesis through controlling the full activation of MAPK signaling pathways. GeGe3 alters angiogenesis by targeting DMPK in tumor endothelial cells and pericytes.

Conclusion: The pyrazolyl-urea GeGe3, a blocker of MAPK and PI3K pathways, strongly inhibits physiological and tumor angiogenesis. In addition, we identified direct targets of GeGe3 including DMPK1, a new angiogenesis target.

Synthesis and complete biological data will be reported in poster session.

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Structural and functional characterization of the GEBR library: selective targeting of PDE4D for cognitive improvement in neurodegenerative diseases

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The inhibition of human phosphodiesterase-4 (PDE4) has been proposed as a strategy for the treatment of several neurodegenerative and inflammatory pathologies (1,2,3,4). Over the last few years, the design of compounds that are selective for specific PDE4 isoforms (mostly PDE4D and PDE4B) has been explored as a way to limit the side effects (emesis and diarrhea) that are associated with unspecific PDE4 inhibition (5,6,7,8). In particular, the so-called GEBR library has been developed in an effort to selectively inhibit PDE4D for cognitive amelioration in Alzheimer's disease (AD) patients. Indeed, some compounds of this library have been shown to have interesting pro-cognitive and memory enhancement properties in AD transgenic mice (5, 6, 7); however, to date no structural data describing their interactions with the enzyme has been made available in the literature. Using a combination of structural biology (X-ray crystallography), biochemistry (enzymatic assays) and *in silico* modelling (molecular dynamics), we set out to address the biochemical behavior of the large GEBR library in order to gain a mechanistic insight into the action of these compounds and pave the way to the rational design of the next generation of inhibitors.
So far, we have solved several high resolution crystal structures of the complex between the PDE4D catalytic domain and the most active GEBR molecules, thus allowing for the detailed identification of the binding mode of each ligand and the precise chemical features that influence its interaction with the target. Among these, the nature of the central moiety plays a crucial role in the conformational freedom of the inhibitor. Based on the different conformations of the ligands, we hypothesize an involvement of the regulatory domains of the enzyme (not present in our crystal structure) as possible interactors. Therefore, we are now investigating the differential inhibition properties of the library between the catalytic domain and the full length enzyme. Moreover, owing to the difficulty in crystallizing the full length version of the enzyme, we are also addressing this issue by simulating the behavior of the whole system by molecular dynamics (MD).
Structure analysis results will be discussed during the poster session.

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A multi-component one-pot synthesis of 3-amino alkylated indoles, new interesting anti-proliferative agents against breast cancer cells

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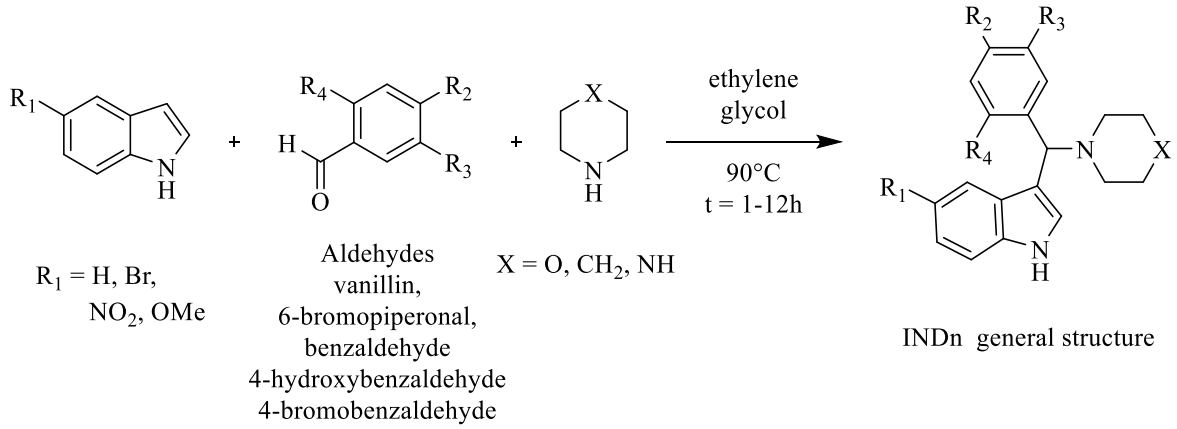
Indole nucleus is a very useful scaffold to develop biologically active molecules, especially anticancer compounds. (1,2) Several indole-based molecules have been synthesized and their antitumor activity evaluated in various cancer cell lines, including breast cancer ones. (3,4) Breast cancer is the most prevalent cancer and the second leading cause of cancer mortality in women with estrogen receptor α-positive (ERα+) disease. (5) Also, the G protein-coupled estrogen receptor-1 (GPER-1) emerged as a useful target to treat the most aggressive triple negative breast cancer. From the medicinal chemist point of view various scaffolds have been already widely studied. A green chemistry approach was followed to variously decorate the 3-aminoalkylated indole. The antitumor activity of the obtained derivatives was evaluated against three different human breast cancer cell lines (MDA-MB-468, MDA-MB-231, SKBR3). All the compounds showed a dose-dependent anti-proliferative effect, in particular against Triple Negative breast cancer cell line MDA-MB-231. Further investigations will identify the mechanism of action and the biological target of the new derivatives.



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Design and synthesis of a new series of indole-based compounds as antitumor agents

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The indole scaffold represents one of the most important structural motifs for the discovery of new biological active compounds (1).
A series of indole-based derivatives was identified as potential antitumor agents, particularly against HeLa cell line. Compound 3-(((2-([1,1'-biphenyl]-4-yl)ethyl)(methyl)amino)methyl)-N-(4-fluorophenyl)-1-methyl-1H-indole-5-carboxamide (compound **1**) showed an interesting cytotoxic effect with an IC_{50} of 0.24 μM at 48h (Figure 1).

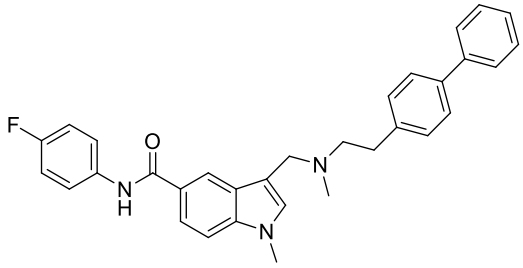

Figure 1: compound **1** (IC_{50} = 0.24 μM).

Starting from compound **1** and aimed to improve the cytotoxic activity, we have recently designed and synthesized a new series of indole-based derivatives to elucidate the structure-activity relationships at the basis of the biological activity.
The structural modifications involve replacement of the:

1) Biphenylethylamine group
2) Methyl group on tertiary amine
3) Methyl group at the N-1
4) Amidic substituent in C-5

References: Vikas Sharma et al. Biological Importance of the Indole Nucleus in Recent Years: A Comprehensive Review *J. Heterocyclic Chem*., 47, 491 (**2010**)

Synthesis and biological activity of new complex polycyclic compounds: autophagy and apoptosis induction

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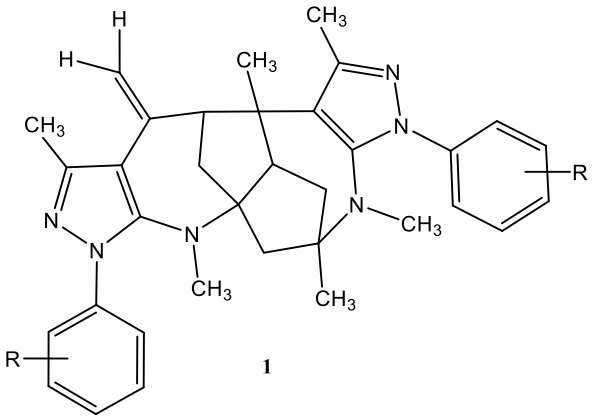
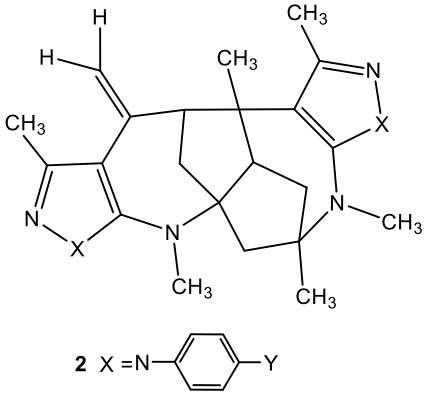
Previously we reported the synthesis of new polycyclic compounds **1** by reacting methylaminopyrazoles and hexane-2,5-dione in 1,4-dioxane in the presence of p-toluensulfonic acid [1]. Some of them resulted be endowed with antiproliferative activity when tested against the NCI panel of human tumoral cell lines. In order to gain more insight on the SAR of this class of compounds, as well as on their mechanism of action, we synthesized the new analogues **2**, **3** and **4**. Compounds **2** bear hydrophilic substituents to each of two phenyl groups, compound **3** bears methyls linked to pyrazole moieties in the place of phenyls, whereas compound **4** contains the isoxazole ring as heterocycle, in substitution of the pyrazole ring. All the above compounds have an increased potential for H bonds formation and/or higher water solubility as compared to compounds 1. Preliminary studies were concerned with the effect of one of compounds **2** (X= OH) on MDA-MB231 cells, a triple negative breast cancer cell line. This compound reduced cell viability in a dose and time-dependent manner, showing an IC_{50} at 48 h of treatment of 12.5 µM. Exploring the biological activity of the compound we demonstrated that this compound causes a G2/M cell cycle arrest at 24-48 h of treatment, followed by a remarkable DNA fragmentation at 48-72 h. Morphological analyses of cells incubated with monodansylcadaverine revealed that the effects of the compound observed in the first phase of treatment are related to the production of dot-like structures and activation of LC-3, two known hallmarks of autophagy. Since autophagy occurred in the first 24 h of incubation with the compound, it probably served as a pro-survival mechanism that was followed by the apoptotic program at 48-72h as demonstrated by chromatin condensation, DNA fragmentation and caspase 9 activation.



1

R = H, 4-CH_3, 3-Cl, 4-Cl, 4-OCH_3



2 X =N–Y

Y = OH, NH_2, $NHCOCH_3$, SO_2NH_2

3 X = N-CH_3

4 X = O

Reference: 1. B. Maggio, D. Raffa, M.V. Raimondi et al., Eur. J. Med. Chem. 72 (2014) 1-9.

Synthesis of a group of novel Xanomeline/77-LH-28-1 hybrid ligands and their FRET investigation at muscarinic acetylcholine receptor subtypes

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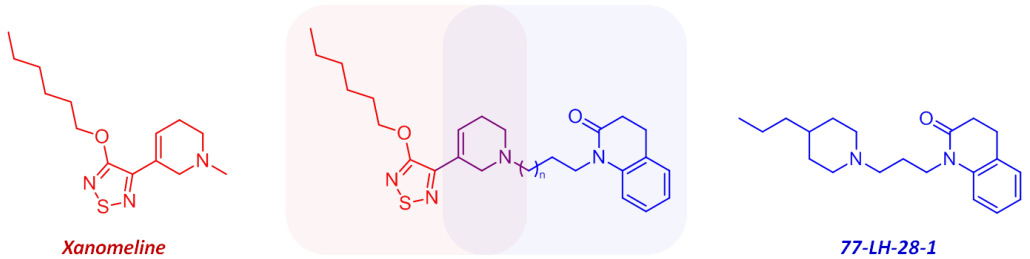
In connection with our interest in investigating novel rationally designed bitopic (i.e., orthosteric/allosteric) derivatives targeting muscarinic acetylcholine receptor (mAChR) subtypes (1,2,3), in this study we designed and synthesized a new set of ligands that integrate in the same molecular skeleton the pharmacophoric moieties of Xanomeline and of 77-LH-28-1 (1-[3-(4-butyl-1-piperidinyl)propyl]-3,4-dihydro-2(1*H*)-quinolinone). Xanomeline is a well-known M_1/M_4-preferring orthosteric agonist, which ameliorated cognitive impairments in Alzheimer's disease patients and showed activity in various models of schizophrenia, thus being potentially beneficial for treatment of positive, negative and cognitive symptoms (4). On the other hand, 77-LH-28-1 was characterized as an M_1-selective, positive allosteric modulator, thus representing an interesting pharmacological tool with cognition enhancing properties (5). As illustrated below, we planned the novel bipharmacophoric derivatives as merged structures, with the tetrahydropyridine nucleus of Xanomeline as the central core.



In the last years, different receptor sensors, based on the fluorescence resonance energy transfer (FRET), were generated for various G protein-coupled receptors, and represented a valuable tool to investigate real time receptor activation as well as ligand-receptor interactions. Recently, this analysis was performed also on a set of bitopic ligands designed for a selective interaction with M_1 mAChRs (6). Our preliminary results on the group of Xanomeline/77-LH-28-1 hybrid compounds indicate, for the M_1 sensor, a reproducible activation response, which depends on the linker length. Conversely, no FRET-related effect could be detected at the M_2 sensor. Thus, a critical spacer length of the hybrid compounds induces conformational changes with a degree of selectively for the M_1 muscarinic receptor. The synthesis and the results of pharmacological investigation will be presented and discussed.

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77-LH-28-1 as a model for the rational design of selective dopamine D_4 receptor ligands

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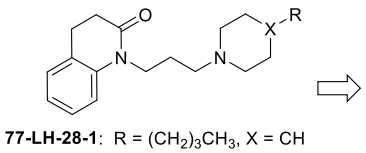
M_1 muscarinic acetylcholine receptor (M_1 mAChR) represents an attractive target for the treatment of cognitive deficits associated with several pathologies, including Alzheimer's disease and schizophrenia. However, the discovery of subtype-selective agonists is hampered by the high degree of homology among the M_1-M_5 mAChR subtypes at the orthosteric binding site. The advent of functional screening assays allowed the identification of ligands, such as 77-LH-28-1, which bound to an allosteric site and selectively activated the M_1 mAChR (1). Initially described as an allosteric agonist by GlaxoSmithKline, at present 77-LH-28-1 is considered a bitopic agonist (2). It displayed antipsychotic and cognition-enhancing efficacy in pre-clinical models of schizophrenia and Alzheimer's disease (1). Unfortunately, its efficacy was confounded by nonselective effects on other receptors (3). Among these receptors, 77-LH-28-1 has been reported to bind the short isoform of the dopamine D_2 receptor ($D_{2S}R$) (4). Dopamine D_2-like subfamily includes D_2R, D_3R and D_4R subtypes. The wide expression of D_2-like receptors in the central nervous system and the modulation of various neurological processes, including gratification, cognition, learning and memory, make them attractive therapeutic targets (5). To get more information about the pharmacological dopaminergic properties of 77-LH-28-1, this compound was evaluated for its affinity at dopamine D_2-like receptor subtypes by radioligand binding assays. Surprisingly, 77-LH-28-1 showed high affinity and selectivity for D_4R over D_2R and D_3R. To better understand the structural features required for the selective interaction with D_4R, the aliphatic butyl chain of 77-LH-28-1 was modified and the novel compounds **1-6** were prepared. Moreover, the piperidine ring of 77-LH-28-1 was replaced by a piperazine nucleus, to give the novel derivatives **7-13** (Figure 1).



77-LH-28-1: R = $(CH_2)_3CH_3$, X = CH

1: R = CH_3, X = CH
2: R = $(CH_2)_2CH_3$, X = CH
3: R = $(CH_2)_4CH_3$, X = CH
4: R = C_6H_5, X = CH
5: R = $CH_2C_6H_5$, X = CH
6: R = $(CH_2)_2C_6H_5$, X = CH

piperidine derivatives

7: R = CH_3, X = N
8: R = $(CH_2)_2CH_3$, X = N
9: R = $(CH_2)_3CH_3$, X = N
10: R = $(CH_2)_4CH_3$, X = N
11: R = C_6H_5, X = N
12: R = $CH_2C_6H_5$, X = N
13: R = $(CH_2)_2C_6H_5$, X = N

piperazine derivatives

Figure 1

All the compounds were evaluated for their affinity at dopamine D_2R, D_3R and D_4R subtypes, as well as at the five mAChR subtypes. Compounds showing the highest affinities at D_4R were also evaluated for their functional activity considering both G-protein activation and β-arrestin recruitment. The most interesting derivatives can be emphasized as biased D_4R compounds, behaving as potent partial agonists for G-protein activation and potent antagonists in β-arrestin recruitment. The detailed results of the biological assays performed to the new derivatives will be reported.

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Design and synthesis of tetrahydrobetacarboline-based derivatives as new TRPM8 modulators

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Transient receptor potential melastatin type 8 (TRPM8) is a transmembrane, nonselective Ca^{2+} permeable cation channel,1 considered as the major sensor for peripheral innocuous cool, and its modulation contributes to a wide range of physiological and pathophysiological processes.(1) One of the most investigated effects produced by TRPM8 modulation is the analgesia against chronic and neuropathic pain: in fact, it has been reported that peripheral and central activation of TRPM8 induces analgesia, specifically reversing the sensitization of the behavioral reflexes elicited by peripheral nerve injury.(2) In the search for TRPM8 inhibitors, we have recently identified two hits bearing a tetrahydrobetacarboline scaffolds (derivatives **1**, **2**). These two small molecules have been characterized both by fluorescence-based and patch-clamp assays. (Figure 1)

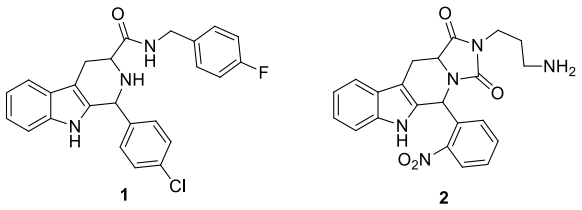

Figure 1. Compounds acting as antagonists of TRPM8

They showed selectivity over TRPM8, lacking of pharmacological activity over TRPA1 and TRPV1 and a potency in the micromolar range. On the basis of these findings, we have designed and synthesized a new library of compounds using a differently decorated tetrahydrobetacarboline motif (figure 2) in the search for a rationale structure-activity relationship and for more potent *lead compound.*

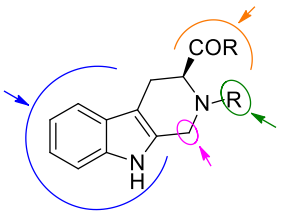

Figure 2. Possible derivatization positions on the tetrahydrobetacarboline motif

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Development of small modulators of protein-protein interactions endowed with anticancer activity

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LxxLL-like motif has been reported as one of the most representative protein-recognition motifs in cell cycle regulation (1, 2). Then, the identification of small molecules able to mime the hydrophobic side chains of the interacting residues of this binding motif is an intriguing task potentially endowing compounds with antiproliferative activity. Recently we have reported the cytotoxic activity of a small set of pyrrole derivatives on different tumor cell lines (MCF7, Huh7, M14, Jurkat) as well as on mouse monocyte macrophages (Raw) cell line (3). Molecular modeling studies carried out on the most active compound of this series (4-benzoyl-5-methyl-1-(4-methylbenzylbenzyl)-1H-pyrrole-2 carboxylic acid 3-chlorobenzylamide), indicated its ability to reproduce the same orientation of the hydrophobic side chains of the *i, i+3, i+4* as well as *i, i+4, i+7* residues in LxxLLxxL-like motifs. Biological studies evidenced the involvement of p53 in its mechanism of action and supported the hypothesis that our lead is a mimetic of *i, i+4, i+7* residues of the p53 ^{19}FxxLWxxL^{26} motif in its interaction with the binding partner MDM2. In order to extend our SAR investigation, a new set of pyrrole-based analogues has been designed and synthesized. The new analogues have been tested on A375 and HTT-116 tumor cell lines taking into account their solubility in PBS buffer, evaluated by Lipinski's approach (4).

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Substituted pyrazolo[3,4-*b*]pyridines as potent A_1 adenosine antagonists

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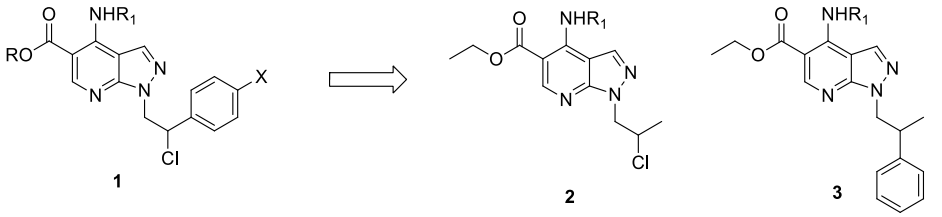
Adenosine is an endogenous neuromodulator which mediates its effects by interacting with four G-protein-coupled receptor subtypes named A_1, A_{2A}, A_{2B} and A_3. These receptors are distributed in a wide variety of tissues, including the central nervous system (CNS), cardiovascular system and airways, where they play key roles in the regulation of several biological functions. Many studies showed that some pathophysiological states are associated with changes of adenosine levels, making the search for adenosine receptor agonist or antagonist an interesting target in medicinal chemistry (1). In particular, an excessive stimulation of A_1 adenosine receptors (A_1ARs) is related to different pathologies, such as various forms of dementia, including Alzheimer's disease, depression, congestive heart failure, bradyarrhythmias and asystolic arrest. For these reasons, many A_1ARs antagonist have been developed in the last decades (2).

In this context, our group synthesized a wide library of 4-aminopyrazolo[3,4-*b*]pyridine-5-carboxylic acid esters **1** active as A_1AR antagonists both on bovine and human receptors; some of these compounds are characterized by high affinity and selectivity towards A_1AR, with the most active compounds having a bovine A_1AR affinity in the low nanomolar range (3). Starting from these promising results, we decided to synthesize a second generation of compounds **2**, with the aim of obtaining more potent and selective agents for human A_1AR. Since previous studies indicated that human A_1ARs contain a binding pocket smaller than that of bovine receptors, we substituted the N1 2-cloro-2-phenylethyl chain with the less bulky 2-cloropropyl chain. Furthermore, to extend SAR evaluations, we synthesized compounds **3** bearing in N1 the 2-phenylpropyl chain. Assays performed on bovine cortical membranes and human A_1AR CHO transfected cells show that compounds **2** are endowed with an improved activity on human A_1AR compared with the first generation derivatives **1**. Derivatives **3**, as expected, show good affinity for bovine A_1ARs, but are less active on human A_1ARs. Biological data will be reported in the poster section.



R = alkyl groups
NHR_1 = aliphatic and aromatic amino groups
X = H, Cl, Br, F

NHR_1 = aliphatic and aromatic amino groups

General structures of first generation (**1**) and second generation (**2** and **3**) of pyrazolo[3,4-*b*]pyrimidines.

References: 1. Chen, J. F. *et al*. Nat. Rev. Drug Discov. 2013, 12, 265-286. 2. Schenone, S. *et al*. Curr. Top. Med. Chem. 2010, 10, 878-901. 3. Tuccinardi, T. *et al*. Chem. Med. Chem. 2008, 3, 898-913.

In Vivo fluorescence imaging of glioblastoma using Translocator Proteins (TSPOs) targeted nanoparticles

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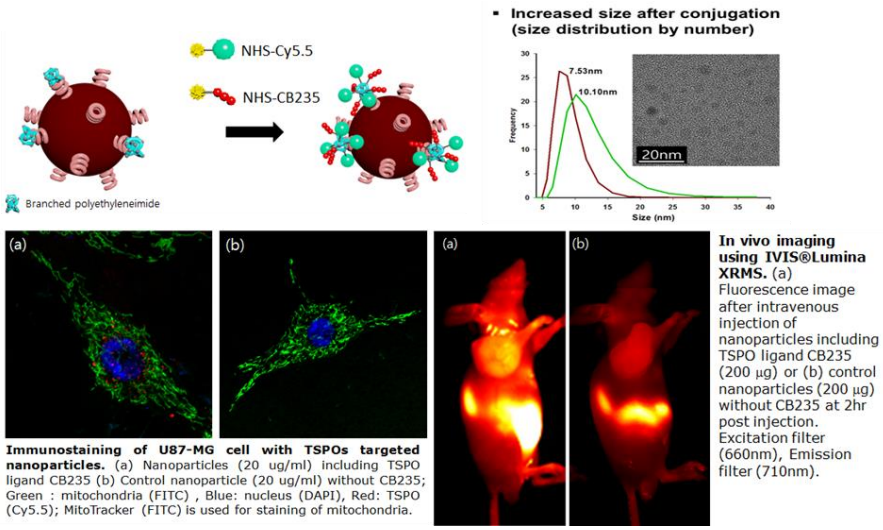
Translocator protein (TSPO) is a five transmembrane domain protein mainly located in the outer mitochondrial membrane. Interestingly, TSPO is overexpressed in a variety of tumors, namely, ovarian cancer, liver tumors, breast carcinoma, colorectal cancer and certain brain tumors such as the glioblastoma multiforme (GBM) and its expression appears to be related to the degree of tumor malignancy (1). GBM is the most common and lethal type of primary brain tumor. In fact, the median survival of GBM patients is less than 16 months despite optimal treatment of currently available therapies. Complete surgical resection of GBM is critical to improve GBM treatment, thus increasing the survival of affected patients. Based on the enhanced expression of TSPO in GBM, the aim of the study was the development of TSPO targeted iron oxide nanoparticles (10.1 nm) using an imidazopyridine based TSPO ligand, namely CB235 (2), and a near-infrared fluorescent dye, specifically Cy5.5, for successful delineation of GBM during surgery. *In vitro* cell imaging experiment showed selective sensitivity of the developed nano-probe for TSPO-rich cell lines including U87-MG human GBM cells, PC-3 human prostate cancer cells instead of CCD-986sk human fibroblasts used as control and characterized by low TSPO expression. *In vivo* experiments conducted on a human GBM U87-MG xenografts animal model proved the specificity of the probe to target GBM. In particular, TSPO targeted nano-probes were compared to non-targeted control nanoparticles (7.53 nm) and showed superior signal-to-noise ratio for GBM. Taken together, the high affinity for TSPO of compound CB235, the passive targeting of nanoparticles also known as Enhanced Permeability and Retention effect and the suitable optical characteristics of near-infrared fluorescent dye for *in vivo* imaging, highlight the possibility of our imaging technique to improve GBM visualization during surgery.



Immunostaining of U87-MG cell with TSPOs targeted nanoparticles. (a) Nanoparticles (20 ug/ml) including TSPO ligand CB235 (b) Control nanoparticle (20 ug/ml) without CB235; Green : mitochondria (FITC) , Blue: nucleus (DAPI), Red: TSPO (Cy5.5); MitoTracker (FITC) is used for staining of mitochondria.

In vivo imaging using IVIS®Lumina XRMS. (a) Fluorescence image after intravenous injection of nanoparticles including TSPO ligand CB235 (200 μg) or (b) control nanoparticles (200 μg) without CB235 at 2hr post injection. Excitation filter (660nm), Emission filter (710nm).

References: 1. Denora N. et al. *Coordination Chemistry Reviews*, **2017**, 341, 1-18. 2. Denora N. et al. *Journal of Controlled Release*, **2013**, 172, 1111-1125.

Novel Hybrid Compounds Dual Targeting GSK-3β and Oxidative Stress for the Treatment of Alzheimer's disease

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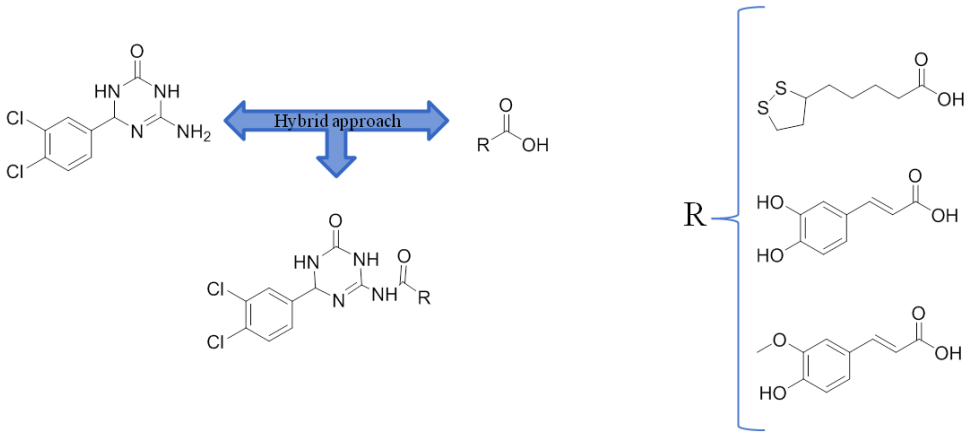
Alzheimer's disease (AD) is a complex multifactorial pathology in which beta amyloid plaques, neurofibrillary tangles and oxidative stress play a fundamental role in the underlying neurodegeneration (1). In this respect, the multi-target-directed ligands (MTDLs) approach could possibly be a more efficient solution to combat the disorder (2). On this basis, the project's purpose was to synthesize novel MTDLs, which can inhibit the GSK-3beta enzyme and at the same time present a strong antioxidant action. Indeed. GSK-3β is a validated target in the tau cascade and reactive oxygen species (ROS) production is another critical player in AD pathogenesis. To achieve this, we fused two chemical scaffolds, i.e. a triazinone and a structure with antioxidant function, as depicted below. The 6-amino-4-(3,4-dichlorophenyl)-3,4-dihydro-1,3,5-triazin-2(1H)-one fragment has been selected because of its reported ability to inhibit GSK-3β at a micromolar level concentration (3). Lipoic, ferulic and caffeic acids have been chosen as the anti-oxidant fragments, thanks to their well-known neuroprotective and ROS scavenging properties (4). By exploiting the carboxylic function of the selected acids, a series of new hybrids has been synthesized through coupling reactions with the amino group in position 6 of the triazinone. To preliminary investigate the anti-AD potential of the synthesized hybrids, we will perform biological assays aimed to test their GSK-3β inhibitory activity and to evaluate their cellular neuroprotective and antioxidant properties.



References: 1. https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-fact-sheet. 2. Bolognesi, ML et al *ChemMedChem.* **2016**, 20;11(12), 1190-2. doi: 10.1002/cmdc.201600161. 3. Prati, F. et al *Angew Chem Int Ed Engl*. **2015**, 26;54(5), 1578-82. doi: 10.1002/anie.201410456. 4. Feng, Y. et al *Oxid Med Cell Longev* **2012**, 17. http://dx.doi.org/10.1155/2012/472932

CLIPS Technology Applied to the Design of Cyclic Peptides with Potent Mixed μ/δ Opioid Activity

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Opiates are widely used in the treatment of pain, but their use is strongly limited by serious side effects such as development of tolerance, physical dependence addiction and respiratory depression. Also, they are not efficacious for the treatment of cronic and neuropatic pain. Recently, it has been demonstrated the existence of physical and functional interactions between the opioid receptors and the formation of omo- and hetero-dymers, like the heterodimeric complex of μ/δ receptors and their contemporary activation might lead to a sinergic and more potent analgesic effect at relatively minimum dose of drug (1-4).

Biphalin (Tyr-(D)Ala-Gly-Phe-NH-)$_2$, a potent mixed μ/δ receptors agonist and DPDPE (Tyr-*c*[(D)Pen-Gly-Phe]-(D)Pen-OH), a refecence cyclic peptide selective for δ receptor have been modifies in order to obtain novel cyclic compounds with improved metabolic stability, potency and in vivo efficacy. Several cyclization approach have been done by our research group and recently we used a different linker in place of disulfide bond by CLIPS approach. The D-Cysteine or D-Penicillamine thiol groups were reacted with three di-bromoylene isomers to close the cycle. The substitution of disulfide bond, which is prone to reduction, with more stable bridges may improve the metabolic stability and the pontency in order to increase the analgesic activity of the new compounds. After designing and synthesis, we evaluated their affinity at the μ and δ opioid receptors by using *in vitro* models like competition binding assays and GTP stimulation assays. All the cyclic compounds showed good affinity for δ and μ opioid receptors. *In vivo* antinociception assays have been also carried out and evaluated with the tail flick test, hot plate test and formalin test. We observed that the DPDPE analogue with p-xylene regioisomer exerted a potent analgesic effect ranging from 15 to 60 min, after i.c.v. and s.c. administration, whereas the most active biphalin analogues was the compound containing the o-xylene bridge. In conclusion, we have obtained two potent compounds, one biphalin and one DPDPE derivatives able to elicit a robust antinociceptive effect in rats both after central and local peripheral administration.

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Efficient antagonists of SMO and GLI1 Hedgehog signaling targets identified by computational screening

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Hedgehog (Hh) signaling is essential for tissue development and stemness. Activating germline or somatic mutations of genes encoding Hh pathway components are found in basal cell carcinoma (BCC) and medulloblastoma (MB), while uncontrolled Hh signaling has been reported to drive tumor progression in several cancers, including lung, breast, stomach, pancreas and hematopoietic malignancies. For this reason, the development of Hh inhibitors is eliciting great interest in drug discovery.(1)

Based on the availability of structural details of SMO and GLI1, which are the most relevant upstream and downstream regulators of the Hh signaling pathway, respectively, we set up a structure-based screening strategy boosted by computational studies. In the case of SMO, the binding site of drugs and drug-candidates is well established and characterized within the heptahelical bundle of the receptor.(2,3) In the case of GLI1, computational and experimental efforts were first spent to clarify the structural requirements of its binding to DNA and to identify a putative ligand binding site.(4) Subsequently, an *in house* library of natural products and their derivatives was screened *in silico* against SMO and GLI1 targets by means of molecular docking, to identify novel Hh inhibitors. A synthetic chalcone derivative emerged as profitable SMO antagonist providing Hh inhibition *in vitro* on cancer (MB and BCC) and cancer stem cells (MB), and *in vivo* (BCC). The molecule proved to inhibit Hh also in the presence of a drug-resistant form of SMO.(5) Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae),(4,5,6) emerged as efficient GLI1 antagonist that binds GLI1 zinc-finger and interferes with its interaction to DNA. Remarkably, GlaB inhibited the growth of Hh-dependent MB and BCC cells *in vitro* and *in vivo*, as well as the self-renewal ability and clonogenicity of MB cancer stem cells.

In summary, computational tools proved highly versatile and reliable in understanding the structural requirements of Hh target proteins and in identifying highly efficient small molecule modulators of pharmacological relevance.

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Discovery of tethrahydro-betacarboline based selective TRPM8 antagonists

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Transient receptor potential melastatin type-8 (TRPM8) is a non-selective Ca^{2+} permeable cation channel activated by cold and the cooling compounds menthol and icilin.(1) An increasing body of evidence suggests that TRPM8 may be an important player in various chronic conditions, such as inflammatory/neuropathic pain and prostate cancer, underscoring its potential as pharmacological target in these pathologies. Recently, we have identified two tryptamine-based derivatives acting as selective modulators of TPRM8 channel (Figure 1).(2)

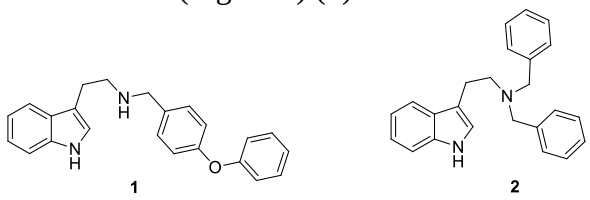

Figure 1: selective activator (**1**) and inhibitor (**2**) of TPRM8 channel.

Tetrahydroisoquinoline-derived ureas (**3**,**4** derivatives, Figure 2) have also been identified as selective modulators of TRPM8.(3) To mimic the spatial arrangement observed in the tetrahydroisoquinoline modulators we decided to apply a conformational restriction to the tryptamine nucleus designing and synthesizing a series of tethrahydro-betacarboline compounds (Figure 2, compound **5**).

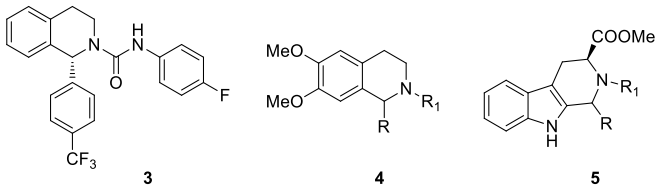

Figure 2: Tetrahydroisoquinoline TRPM8 modulators (3,4) used as starting point for design new tetrahydrobetacarboline

These compounds were tested as TRPM8 modulators by fluorescence and electrophysiology-based (patch-clamp) assays. As a result of preliminary fluorescence-based screening assay, we identified two compounds acting as inhibitors of calcium influx in HEK293 cells, stably expressing TRPM8 channels, with IC_{50} values of 10.7±0.6 μM and 30.3±0.7 μM respectively.

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New pyrrole inhibitors of chronic myeloid leukemia cell growth

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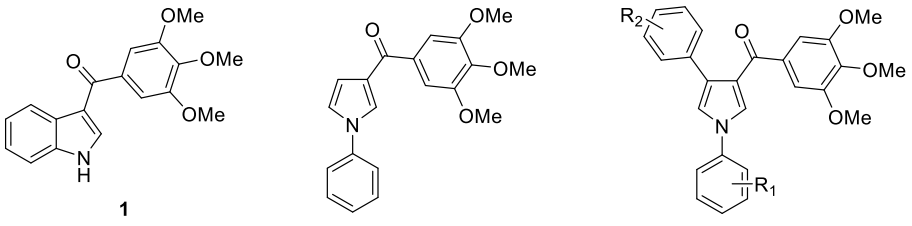
Microtubules are an attractive target for the development of effective anti-leukemia agents.[1] Evidence has accumulated correlating inhibition of tubulin polymerization and leukemic cell proliferation.[2] The activity of colchicine site agents in chronic myeloid leukemia (CML) has not been adequately explored.

Recently, starting from previously reported aroylindoles (ARI, **1**)[3] we developed a class of 3-aroyl-1-arylpyrroles (ARAPs, **2**) via benzocracking approach by shifting the indole benzene moiety to position 1 of the pyrrole ring.[4] ARAPs proved to be potent inhibitors of both tubulin assembly and cancer cells growth, by binding the colchicine binding site. Pursuing our studied on tubulin targeting agents, we designed 3-aroyl-1,4-diarylpyrroles (ARDAPs, **3-16**) as potential anticancer agents bearing different substituents at the 1- or 4-phenyl ring (Chart 1).

ARDAPs exhibited potent inhibition of tubulin polymerization, binding of colchicine to tubulin and cancer cell growth. (4-(4-Aminophenyl)-1-phenyl-1*H*-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)methanone inhibited the proliferation of BCR/ABL-expressing KU812 and LAMA84 cells from CML patients in blast crisis and of hematopoietic cells ectopically expressing the imatinib mesylate (IM)-sensitive KBM5-WT or its IM-resistant KBM5-T315I mutation. The same compound minimally affected the proliferation of normal blood cells, indicating that it may be a promising agent to overcome broad tyrosine kinase inhibitor resistance in relapsed/refractory CML patients. New ARDAP significantly decreased CML proliferation by inducing G2/M phase arrest and apoptosis via a mitochondria-dependent pathway and increased the cytotoxic effects of IM in human CML cells.

Chart 1. Chemical structures of ARI (**1**), ARAP (**2**) and ARDAP (**3-16**) derivatives.



R_1, R_2 = H, 2-F, 3-Me, 3-F, 3-NH_2, 4-Me.

References: (1) De Bruin, E. C.; Medema, J. P. *Cancer Treat. Rev.* **2008**, *34*, 737-749. (2) Bates, D.; Feris, E. J.; Danilov, A. V. et al. *Cancer Biol. Ther.* **2016**, *17*, 291-299. (3) La Regina, G.; Sarkar, T.; Bai, R. et al. *J. Med. Chem.* **2009**, *52*, 7512-7527. (4) La Regina, G.; Bai, R.; Coluccia, A. et al. *J. Med. Chem.* **2014**, *57*, 6351-6552.

Rational design of new potent non-nucleoside inhibitors of terminal deoxynucleotidyl transferase active in leukemic cells

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Mammalian terminal deoxyribonucleotidyl transferase (TDT) catalyzes the non-template-directed polymerization of deoxyribonucleoside triphosphates and has a key role in V(D)J recombination during lymphocyte and repertoire development. Elevated TDT activity is showed in leukemic cells of acute lymphocytic leukemia and in the chronic myelogenous leukemia crisis. This finding is connected to a poor prognosis and response to chemotherapy. DNA polymerase lambda (Pol λ), homolog to TDT,[1] can synthesize DNA in a template-independent pathway. Pol λ might be involved in the nonhomologous end joning (NHEJ) recombinational repair pathway of DNA double strand breaks. During a random screening on various polymerases we found some aryl diketo hexenoic acids (DKHAs) (RDS 2119, RDS 2153, RDS 2184) (see figure 1), previously synthesized by us as anti-viral agent, as hits showing interesting activity against mammalian terminal deoxyribonucleotidyl transferases.[2,3]

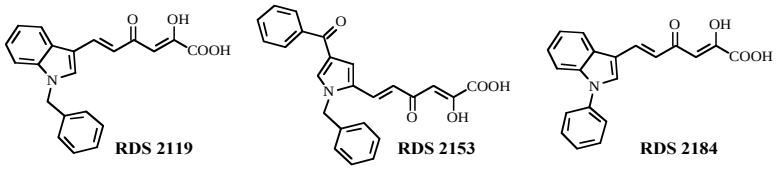

Figure 1. Hit compounds obtained from random screeeng.

Thus, we started SAR studies on DKHAs and found compounds that specifically target TdT behaving as nucleotide-competitive inhibitors.[4] These compounds showed a selective toxicity toward MOLT-4 overespressing TdT, compared to HeLa cells, that well correlate with in vitro selectivity for TdT. The binding site of two of these inhibitors was determined by cocrystallization with TdT, explaining why these compounds are competitive inhibitors of the deoxynucleotide triphosphate (dNTP). These studies opened the possibility to the rational design of TdT inhibitors. Starting form the observed binding pose of inhibitors cocrystallized within the catalytic core, we noted that the phenyl substituent or the benzyl group on pyrrole ring could occupy two different pockets. Thus, we decidesd to design and synthesize compounds bringing two aryl moieties. The design, synthesis and biological assays performed on newly synthesized compounds will be reported and discussed.

References: 1 Hübscher, U.; Maga, G.; Spadari, G. *Ann. Rev. Biochem*. **2002**, *71*, 133-163. 2 Locatelli, G. A.; Di Santo, R.; Crespan, E.; Costi, R. *Mol. Pharmacol.* **2005**, *68*, 538-550. 3 Di Santo, R.; Maga, G. *Curr. Med. Chem*. **2006**, *13*, 2353-2368. 4 Costi R., Cuzzucoli Crucitti G., Pescatori L., Messore A., Scipione L., Tortorella S., Amoroso A., Crespan E., Campiglia P., Maresca B., Porta A., Granata I., Novellino E., Gouge J., Delarue m., Maga G. and Di Santo R. *J. Med. Chem.*, **2013**, 56(18), 7431-7441.

Comparative study of Chitosan and PLGA polymeric nanoparticles containing cidofovir

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Cidofovir (CDV) is a small molecule anti-viral drug and has been used as a local intravitreal injection for viral retinitis (1) to avoid systemic complications. Most formulations, containing cidofovir for intravitreal administration, caused retinal toxicity and visual discomfort in some patients. The nanoparticles seem to be a favorable drug carrier due to its low risk on hindering normal vision and to decrease repeated injections needed in chronic ophthalmic pathologies.
In this study nanoparticles constituted by chitosan (CHI) and poly(D,L-lactic-co-glycolicacid) (PLGA) were compared.
The CHI nanoparticles, with diameter ranging from 200 to 300 nm (approx. 400 nm after redispersion in water), were obtained by ionotropic gelation between CDV and the mucoadhesive polymer chitosan (CHI), using a fractional factorial experimental design to investigate the influence of the some selected variables on the formation of chitosan nanoparticles. While the PLGA nanoparticles, with size around 200-250 nm, were prepared by different emulsion solvent diffusion techniques to reach the optimized formulation.
Both formulations have been characterized by particle size, polydispersion index and zeta potential using a photon correlation spectroscopy (PCS) assembly(Zetasizer 3000 HS).
The CHI/CDV nanoparticles showed a zeta potential value of 30 mV, an encapsulation efficiency about 20% w/w and a yield of 15% w/w. The PLGA/CDV nanoparticles had a zeta potential value of -15 mV, an encapsulation efficiency about 21% w/w and a yield of 40% w/w.
Furthermore, stability studies in water have also been carried out both on the freshly prepared sample and on the centrifuged. The two formulations showed good stability at 24h and 7 days, not significantly chancing the particle size.
Finally, we compare the two analytical methods (UV and HPLC) used to quantify Cidofovir in the sample. By comparing the encapsulation efficiency data of the different nanoparticles, the two analytical methods are both available for the active ingredient dosage since the obtained values are practically the same.
Therefore, for the same quality of analytical technique, we can choose the most convenient in terms of money and time, therefore the choice falls on UV spectrophotometry.

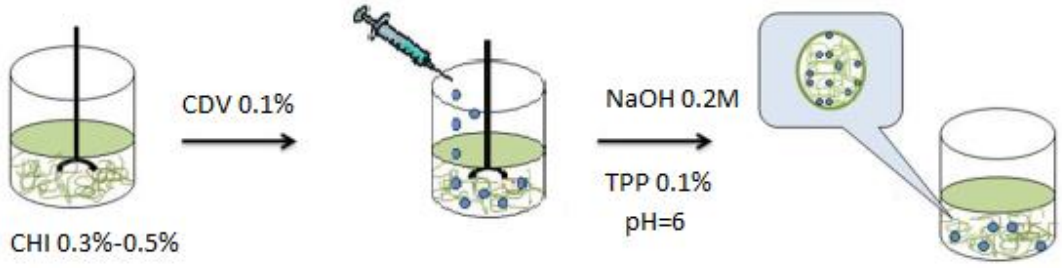

Figure 1: Scheme of CHI nanoparticles preparation containing CDV.

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Synthesis of Novel Benzylpiperazine Derivatives as Ligands for the σ_1 Receptor

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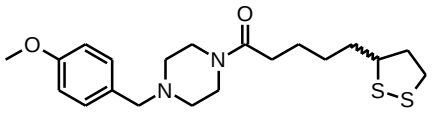
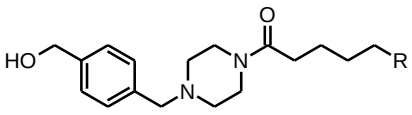
Sigma receptors are classified into two subtypes, sigma-1 (σ_1) and sigma-2 (σ_2) receptor. They are distinguished by molecular weight, anatomical localization, and transduction mechanism. Unlike σ_2 receptor, the σ_1 subtype has been cloned in different species and recently the crystal structure of the human protein has been elucidated (1). The σ_1 receptor is widely distributed in both CNS and peripheral human tissues where it works as molecular chaperone. It regulates activity of many neurotransmitter systems and represents a potential therapeutic target in a number of pathologies, including neurodegenerative diseases, neuropathic pain, and cancer (2).
Along the years, several σ_1 ligands have been discovered; in particular, in a study of Prezzavento *et al*. (3), the 4-methoxybenzylpiperazinyl derivative **A** was reported as a good and selective ligand for the σ_1 over the σ_2 receptor (σ_1 K_i = 5.7 nM; σ_2 K_i = 2460 nM; K_i σ_2/ K_i σ_1 = 432). In this compound, the σ_1 binding property is coupled to an antioxidant activity given by the 1,2-ditiolan-3-yl moiety. Using compound **A** structure as a template and with the aim to obtain new selective σ_1 ligands, a number of novel derivatives (**B** and **C)** were designed and synthesized. These compounds fulfil the Glennon's σ_1 receptor pharmacophoric model in which the essential features for binding are represented by two distal hydrophobic regions and a central positive ionizable group. In derivatives **B**, the methoxy group in **A** was modified in order to explore the importance for binding of an additional H-bond donor group. On the other hand, in compounds of **C** type, the 4-methoxybenzylpiperazinyl moiety was maintained and modifications were carried on the other distal hydrophobic region, varying its nature and length. Synthetic pathways to title compounds along with their complete binding properties will be given at the meeting.

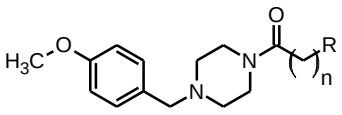


A



B

R = C_6H_5, 1,2-ditiolan-3-il



C

n = 1 - 4; R = C_6H_5, C_6H_{11}
n = 3; R = O-C_6H_5
n = 0; R = HC=CH-C_6H_5

References: 1. Schmidt H. R. *et al*., Crystal structure of the human σ_1 receptor. *Nature* 532, 527-530 , 2016. 2. Su T.-P. *et al*., The sigma-1 receptor as a pluripotent modulator in living systems. *Trends Pharm. Sci*. 37, 262-278, 2016. 3. Prezzavento O. *et al.,* Design and synthesis of new bifunctional sigma-1 selective ligands with antioxidant activity. *J. Med. Chem.* 56, 2447-2455, 2013.

Synthesis of a new generation of pyrazolo[3,4-*d*]pyrimidines as Fyn inhibitors

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Fyn is a member of the Src-family of non-receptor tyrosine kinases (TKs) and it phosphorylates a variety of target proteins involved in several signaling pathways (1).
To date, the involvement of Fyn in solid and in hematologic malignancies has become more evident and its abnormal activity has been shown to be related to severe central nervous system pathologies, such as Alzheimer's and Parkinson's diseases (2).
Our group synthesized different libraries of pyrazolo[3,4-d]pyrimidines **1** (in the **Figure**) active as c-Src (3), and/or Bcr-Abl (4) inhibitors.
Since Src and Fyn possess similar structures, we decided to investigated if some compounds of our libraries are also active as Fyn inhibitors and, at the same time, we synthesized other analogues of compounds **1**. In particular, compounds **2a,b** (in the **Figure**), bearing a 2-chloro-2-phenylethyl chain in N1, an aromatic group in C3 and a primary amino group in C4, possess high activity toward Fyn, inhibit the phosphorylation of the protein Tau in an Alzheimer's model cell line and show antiproliferative activities against different cancer cell lines (5).
On the basis of these interesting results, we decided to expand the structure-activity relationship studies on this family of inhibitors and we planned the synthesis of compounds **3** (in the **Figure**) bearing in N1 the same chain of compounds **2a,b** and different aromatic groups in C3.
Enzymatic assays on these compounds have demonstrated that these molecules are active towards Fyn. Biological data will be reported in the poster section.

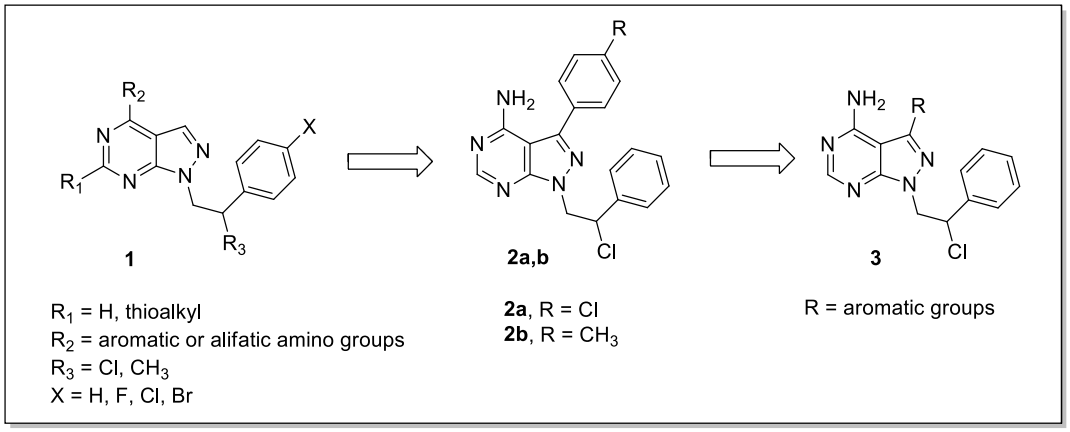

Figure. General structure of our library of pyrazolo[3,4-*d*]pyrimidines **1** and structures of compounds **2a,b** and **3**.

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Highly potent dual acting A_1 and A_3 adenosine receptor ligands: synthesis, binding, functional assays and analgesic effects in mice

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Adenosine (Ado) is a purine nucleoside endowed with many different physiological and pathological functions. Many studies support the fact that Ado acts as a neurotrasmitter and neuromodulator, and as an endogenous agonist on adenosine receptors (ARs). ARs belong to the superfamily of G-protein-coupled receptors (GPCRs) and are represented by four subtypes: A_1, A_{2A}, A_{2B}, and A_3 ARs (1). They are found in almost all kind of tissue: central nervous system (CNS), peripheral neurons, cardiovascular system, respiratory tract and immune system (2). Due to the wide distribution of ARs throughout the body, there is a substantial possibility that Ado ligands will have unwanted effects in non target tissues.

One way to overcome adverse effects is the use of multitarget drugs (3). A multitarget drug may display an improved therapeutic efficacy compared to a highly selective one. In fact, multitarget activities may potentiate the effect of treatment either additively or synergistically. Moreover, a multitarget drug has the advantage of following only one pharmacokinetic and metabolic pattern, thus overcoming the limits of combination therapy.

Substitutions at both purine and sugar moiety of adenosine results on AR ligands endowed with different affinity and selectivity at the four AR subtypes (4). Potent and highly selective A_1AR agonists have been previously obtained by replacement of the 5'-hydroxyl group with a chlorine atom in N^6-substituted-adenosine derivatives (5). 5'-Chloro-5'-deoxy-N^6-(±)-(*endo*-norborn-2-yl)-adenosine (5'Cl5'd-(±)-ENBA) showed analgesic effects in mice without affecting cardiovascular and motor functions (6).

Combining a 5'-*C*-ethyltetrazol-2-yl group with the appropriate N^6-substitution in adenosine derivatives led to an increased affinity versus both hA_1AR and hA_3AR, reaching subnanomolar values, while remaining agonists at hA_1 and antagonists at hA_3AR (7).

In this work a new series of 5'-*C*-ethyltetrazol-2-yl-N^6-substituted adenosine derivatives were synthesized and studied both *in vitro* in binding and functional assays and *in vivo* in a mouse model of pain. Through an in silico receptor-driven approach, the molecular bases of the hA_1- and hA_3AR recognition and activation of this series of 5'-*C*-ethyl-tetrazolyl derivatives were explained.

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Design, synthesis and biological evaluation of N^6/5'-disubstituted adenosine derivatives as A_1 adenosine receptor agonists

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Adenosine is an endogenous purine nucleoside that modulates a variety of physiological functions as a result of its activation of specific G protein-coupled receptors defined as A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs) (1).

The A_1 adenosine receptor (A_1AR) is the best characterized adenosine receptor subtype. Selective A_1AR agonists mediate neuro- and cardioprotective effects, reduce lipolysis in adipose tissue, and intraocular pressure in glaucoma (1,2). The A_1AR is abundantly expressed in spinal cord and other neuronal tissue, and its activation produced pain-relieving effects in a number of preclinical animal models (3). Our previous works discovered that combining the appropriate 5′- and N^6-substitution in adenosine derivatives, highly selective human (h) A_1AR agonists (4) or highly potent dual hA_1AR agonists and hA_3AR antagonists can be obtained (5). The substitution of OH at the 5′-position of N^6-substituted adenosine derivatives with a chlorine atom is not only well tolerated by the hA_1AR but even improves the A_1AR selectivity and affinity. 5′-Chloro-5′-deoxy-N^6-(±)-*endo*-norbornyl-adenosine (5′Cl5′d-(±)-ENBA) turned out to be a potent and the most selective human and mouse (m) A_1AR agonist *vs* A_3AR so far known (4,6) with analgesic effects in a mouse model of neuropathic pain (7). Moreover, it was found to reduce the dyskinesia caused by L-DOPA in a mouse model of Parkinson disease (PD) (8) and the tremor in a harmaline-induced model of essential tremor (ET), suggesting that A_1AR may be a potential target also for the treatment of ET (9).

In order to explore novel combinations of 5′-modification and N^6-substitution leading to potent and selective A_1AR agonists, a series of 5′,N^6-disubstituted adenosine derivatives was synthesized and evaluated for affinity and selectivity at all cloned hAR subtypes.

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Design, synthesis, and biological evaluation of lactam-constrained PTPRJ-binding peptides

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PTPRJ is a receptor protein tyrosine phosphatase whose expression is drastically reduced in the majority of cancer cell lines. PTPRJ is able to interact and dephosphorylate numerous receptor tyrosine kinases (RTKs) whose aberration in tumor cells is responsible of self-sufficiency cell growth, the first hallmark of cancer.(1) In this context, we recently identified PTPRJ-19, [CHHNLTHAC] (fig. 1A), a disulfide bridged nonapeptide, as a positive modulator of PTPRJ.(2)
As part of a wide research program aimed to the identification of new PTPRJ-targeted antitumoral agents, we considered PTPRJ-19 a valuable starting point to clarify the structural elements that are responsible for its interaction with the biological target.
First, in order to study the chemical nature of the bridge and the structural importance of the ring size, we replaced the disulfide bridge by a side chain-to-side chain lactam bridge, a chemically more stable moiety. So we present the synthesis, the conformational properties and biological activities of new cyclic analogues of PTPRJ-19. Results obtained show that lactam cyclic peptide 7 (fig. 1B) is the most active of the synthesized series.

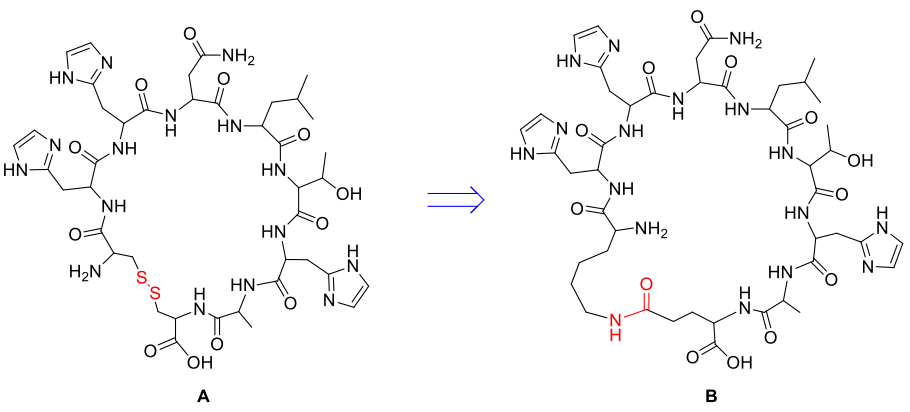

Figure 1. *Structure of PTPRJ 19 (A) and peptide 7 (B).*

References: 1. Keane M.M. et al. *Cancer Res.* **1996**, 56(18), 4236-4243. 2. Paduano F. et al. *ACS Chem. Biol.* **2012**, 7, 1666−1676. 3. Grieco P. et al. *Bioorg. Med. Chem.* **2002**, 10, 3731–3739.

Orienting the design and synthesis towards sigma-2 receptor subtype

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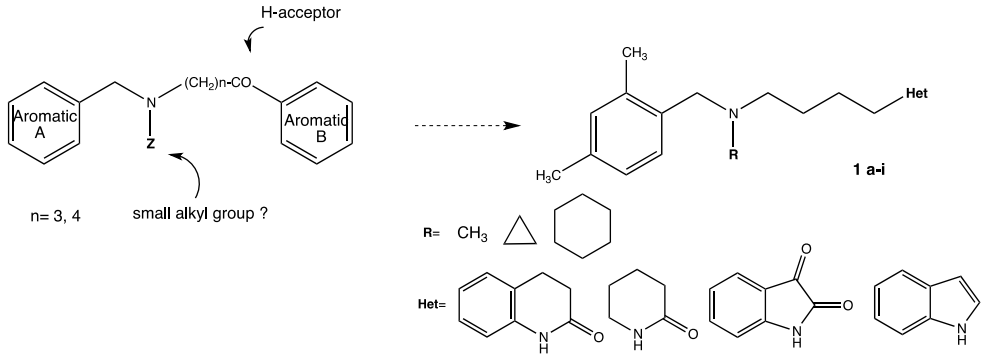
Sigma-2 receptor (σ2R) subtype is definitely an enigmatic kind of receptor and it has not been cloned yet, remaining an unknown protein. σ2Rs are overexpressed in several tumor cells and can be considered a tool for cancer therapy and diagnosis (i.e. PET and SPECT), indeed, it's well-known that σ2R-agonists promote apoptosis leading to cell death. In 2010, our group developed a new σ2R pharmacophore model based on some benzoxazolone derivatives (1). To date, our efforts are focused to discovering new, more selective σ2 ligands and the purpose is to recognize which features are strictly necessary to drive the selectivity through σ2R subtype, considering that most of the compounds present in the literature, and gifted with σ2R affinity, are often structurally different from one another so there's a need to identify the common features to drive the selectivity through σ2R subtype. Relying on some new derivatives bearing different heterocyclic moiety, we found that one of two aromatic fragments (aromatic-B), usually present in σ1R ligands and necessary for their σ1 affinity, can be replaced with a hydrophobic-aliphatic bulky group, as well as the common hydrogen-acceptor function, such as the carbonyl, may be lacking or even reinforced by a further group, still retaining the σ2R affinity. Moreover, we found that the 2,4-dimethyl-substitution on the Aromatic (-A) ring results ideal for the σ2 profile, whilst bulky groups, linked to the basic nitrogen atom such as cyclopropyl or cyclohexyl, adversely affect the σR affinities.



From the data obtained, indeed, we found that an aliphatic group as the piperidin-2-one moiety, still retain the σ2 affinity but drastically reduce the σ1 affinity (K_iσ1 >10000 nM, K_iσ2= 337 nM, σ1/σ2> 30). Same considerations regarding the presence of a further carbonyl function (indoline-2,3-dione; K_iσ1= 8600 nM, K_iσ2= 252 nM, σ1/σ2= 34) or its lack (indole; K_iσ1= 1300 nM, K_iσ2= 440 nM, σ1/σ2= 3).

In conclusion, our present goal is to expand the library of derivatives having features mentioned above in order to generate a new, more reliable, pharmacophore model for the σ2R subtype useful for the research in the field for the development of new anticancer drugs.

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Is it possible to speed-up the discovery of multi-targeting bioactive compounds?

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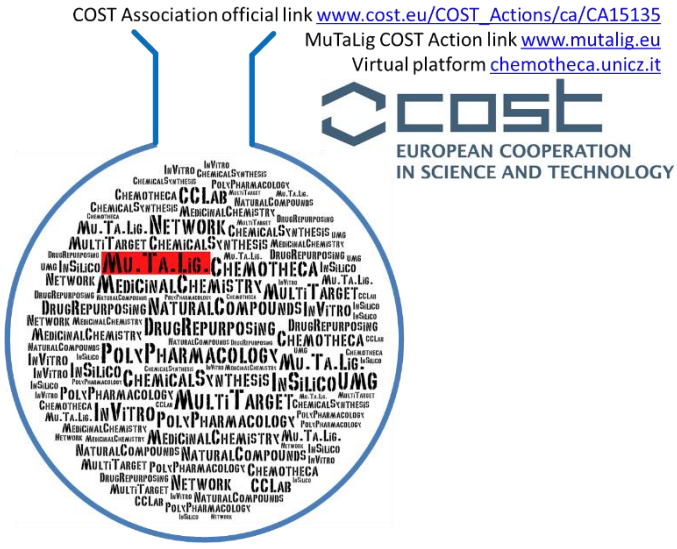
Nowadays, our world is affected by relevant social diseases based on multi-factorial variables. Medicinal chemists are fully engaged to find out novel therapeutic tools against them. The "lock-and-key" theory, introduced by the Nobel prize Paul Ehrlich, is the original approach to identify novel bioactive compounds, by the "one-drug one-target" paradigm. Recently, another trend is overcoming this approach to take better into account the multiple nature of the diseases: the "one drug multiple targets" paradigm. It is based on the capability of bioactive compounds to interact selectively with 2 or more macromolecular targets, exerting their effects against certain therapeutic goals in a synergic manner. (1) This innovative concept prompted in 2015 the creation of a COST Action on this topic among European research groups involved in several chemical and biological areas both at academic and industrial level.(2) For Pharma/Biothec companies this approach can fit with the repurposing issue applied to the multi-targeting and poly-pharmacology, since many bioactive compounds, obtained by means of consistent scientific investigations, could be revaluated and eventually have a new future.



In this communication an answer to the question posted in the title will be proposed and discusses, taking into account the purposes of the COST Action MuTaLig (Multi-target paradigm for innovative ligand identification in the drug discovery process) at the beginning of its second grant year. MuTaLig started with 5 co-proposing European research teams and recently expanded to more than 30 countries.

References: 1. Medina-Franco, JL et al *Drug Discovery Today*, **2013**, 18, 495-501. 2. www.calameo.com/read/003272336045b0302678c

The Molecular dYnamics SHAred PharmacophorE (*MYSHAPE*) approacha new tool to arise docking and pharmacophore modeling performance: virtues and vices

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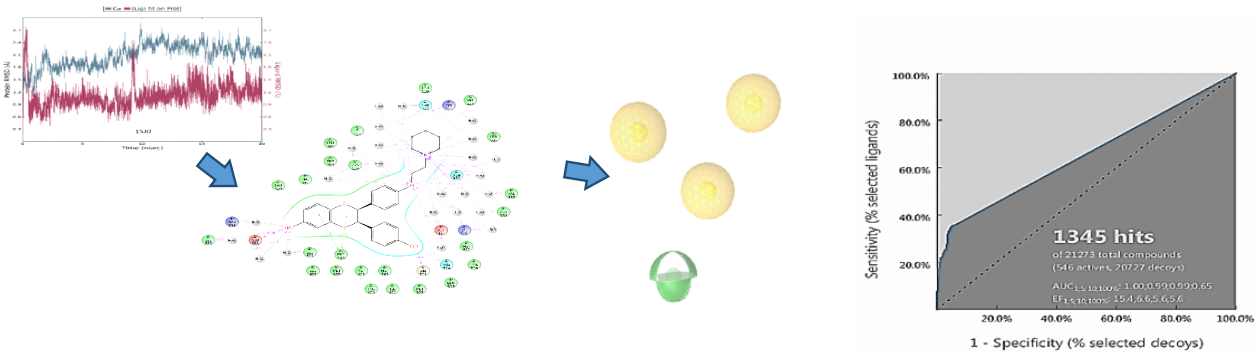
In a recent paper, we presented a new virtual screening workflow that addresses the arising issues of molecular docking and pharmacophore modeling when using a single set of coordinates and a single active ligand [1]. MD simulations were carried out and ligand-protein interactions were analyzed and collected together with their appearance frequency. A pharmacophore model was then created using only the common feature patterns that all the ligands exhibited during MD simulations. This 'Molecular dYnamics SHAred PharmacophorE' was then used for virtual screening on active and inactive molecules library. MYSHAPE was also used as constraints for the creation of the docking grid. The application of the MYSHAPE model showed an interesting increase of the screening capability both in terms of sensitivity of the model and specificity when compared to the PDB models. This work [1] was a first essay for a workflow that should be applied to different proteins. In the present study we tried to apply the MYSHAPE approach to other three different ligand-protein systems (ERα; RXRα, and MAPKp38) with the aim to optimize the method to each different biological target taking in consideration the early recognition. The obtained results for these new targets confirmed that it is mandatory, to optimize the virtual screening campaign, the selection of dynamic features and constraints for docking. In particular, the addition of the constraints derived from MD simulation leads to an improvement in the model selectivity for RXRα and ERα in standard precision docking mode. For MAPKp38, validation metrics such as ROC, BEDROC, and AUAC are higher in extra precision mode. For the pharmacophore modeling, the addition of the features derived from the common interactions in MD simulations guarantee an improvement in the AUC for RXRα (37%), and ERα (77%), but light improvement for MAPKp38.



MD simulation derived common interactions revealed fundamental for docking selectivity, while they are applied to pharmacophore modeling only when the number of final features in the common and dynamic pharmacophore is higher than the starting static pharmacophore. The strength behind the protocol is the ease of use related to the improvement of results. It also could represent a valid alternative to use very time-consuming techniques such as XP docking with constraints.

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Intramolecular oxidative deselenization of acylselenoureas: a facile synthesis of benzoxazole amides and carbonic anhydrase inhibitors

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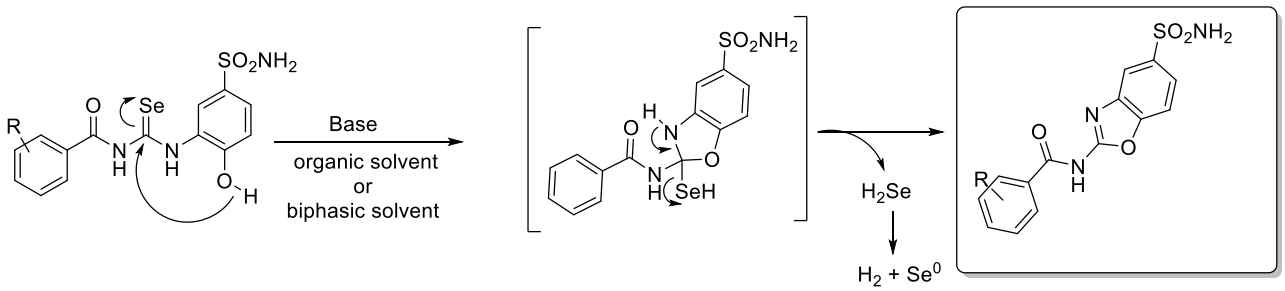
Natural Products (NPs) have an unmatched chemical diversity, and that makes them an attractive source of new compounds for the development of new and more effective drugs. In this context NPs containing the benzoxazoles moieties are of particular interest as they occur in various structurally complex biologically active NPs and possess interesting antibiotic, anti-inflammatory, antihistaminic properties. (1)

Here we report for the first time a mild and efficient synthetic method to convert acylselenoureido derivatives, bearing the O-substituted phenolic moiety, into benzoxazole amides. Mechanistic investigations account for a pH dependent intramolecular cyclization (Scheme 1). (2)

The new synthetic strategy was used to obtain a small series of inhibitors of the metalloenzyme Carbonic Anhydrase (CA; EC 4.2.1.1), by means of introduction of the primary sulphonamide (-SO2NH2) moiety at position 5 of the benzoxazole scaffold, and their enzymatic activity was assessed by means of in vitro kinetic assays.

Since CAs (of the human type or expressed in prokaryotic organisms) are validated pharmacological targets, the new synthetic strategy herein reported opens new insights into the development of NPs containing the benzoxazole ring as effective CA modulators.



Scheme 1: Synthetic method to convert acylselenoureido compounds in benzoxazole derivatives

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Iminothioethers as a novel class of H2S-donor: gasotransmitter release and vascular effects

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Hydrogen sulphide (H_2S) is now considered an important gasotransmitter exerting a plethora of effects, in particular in controlling the homeostasis of the cardiovascular system. Endogenous H_2S is mainly produced in various mammalian tissues by specific enzymes, such as cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE) responsible for metabolizing L-Cysteine (L-Cys). Blunted levels of endogenous H_2S have been found in animal models of many pathological conditions, such as myocardial ischemia, spontaneous hypertension and hypoxic pulmonary hypertension. Therefore, the administration of exogenous H_2S may represent an attractive pharmacological strategy.

The administration of excessively rapid H_2S donors, such as NaHS, is not suitable for clinical purposes. In contrast, organic molecules that are endowed with slow H_2S releasing properties may have a relevant clinical usefulness. (1, 2)

We have recently described a number of arylthioamides characterized by slow and L-cysteine-dependent H_2S-releasing properties. (3) A compound from this class resulted able to strongly abolish the noradrenaline-induced vasoconstriction in isolated rat aortic rings and hyperpolarize the membranes of human vascular smooth muscle cells in a concentration-dependent fashion; in addition, a significant reduction of the systolic blood pressure of anesthetized normotensive rats was observed after its oral administration.

Pursuing our interest in this field, a small library of iminothioethers was synthesised and their H_2S-releasing properties were evaluated in vitro, by amperometric detection, both in the absence and in the presence of organic thiols, such as L-Cys. Furthermore, their vasorelaxing properties were assessed in rat aortic rings. Compounds which exerted the better H_2S releasing properties were selected for further pharmacological evaluation by electrophysiological, spectrofluorimetric and confocal microscopy studies.

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Monitoring peptides released after gastro-intestinal digestion by online comprehensive LC × UHPLC-HRMS: A case study on buffalo milk dairy products

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Dairy products contain many bioactive peptides that are encrypted in the sequence of precursor proteins and become bioaccessible and active after release during gastro-intestinal digestion (1). The resulting matrix are often very complex, containing hundreds of compounds. Conventional analytical techniques based on monodimensional liquid chromatography methods coupled to mass spectrometry are not capable to handle this challenging samples and thus high peak capacity values are necessary (2). In this regard we developed an online comprehensive two dimensional liquid chromatography platform by two coupling two reversed phase columns operating at different pH values (3). In the first dimension a microbore RP column was employed whereas in the second dimension two different short sub-2 μm stationary phases were compared: a fully porous monodisperse C18 column and a core-shell C18 column (4). The peptides were monitored by UV detection and identified by tandem mass spectrometry (MS/MS). The developed method provided double peak capacity values with respect to monodimensional methods and high orthogonality, together with a major number of identified peptides and a quick visualization of matrix differences by 2D map comparison. The method is highly suitable for peptidomics studies (5).

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Novel sulfamide containing compounds as selective Carbonic Anhydrase I inhibitors

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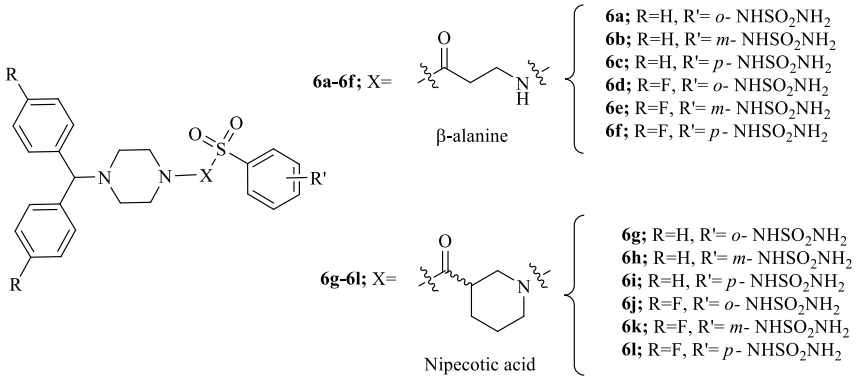
The development of isoform selective inhibitors of the carbonic anhydrase (CA; EC 4.2.1.1) enzymes, represents the key approach for the successful development of druggable small molecules useful for the treatment of human diseases, such as glaucoma, oedema, central-nervous-system (CNS) affecting pathologies, obesity as well as hypoxic cancers (1, 2)
Here, and in agreement with the tail approach, (1) we report a series of new sulfamide derivatives (-NH-SO2NH2) as isosteres of the conventional and most investigated class of inhibitors of these enzymes: the primary sulfonamides (-SO2NH2). All the compounds reported were investigated in vitro for their ability to inhibit in vitro the physiological most relevant human (h) CAs such as I, II, IV and IX. hCA I resulted the most inhibited isoform, whereas all the remaining isoforms showed different inhibition profiles.



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Design and synthesis of novel Nonsteroidal Anti-Inflammatory Drugs and Carbonic Anhydrase Inhibitors Hybrids (NSAIDs−CAIs) for the reatment of rheumatoid arthritis

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We report the synthesis of a series of hybrid compounds incorporating 6- and 7-substituted coumarins (carbonic anhydrase, CA inhibitors) and clinically used NSAIDs (indomethacin, sulindac, ketoprofen, ibuprofen, diclofenac, ketorolac, etc., cyclooxygenase inhibitors) as agents for the management of rheumatoid arthritis (RA) (**Fig.1**). Most compounds were effective in inhibiting the RA overexpressed hCA IX and XII, with KI values in the low nanomolar-subnanomolar ranges. The antihyperalgesic activity of such compounds was assessed by means of the paw-pressure and incapacitance tests using an in vivo RA model. Among all tested compounds, the 7-coumarin hybrid with ibuprofen showed potent and persistent antihyperalgesic effect up to 60 min after administration.[1]

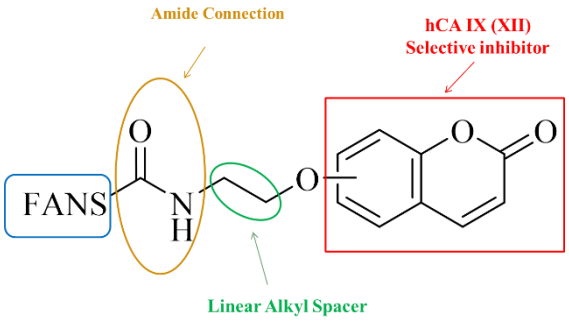

Fig.1: Rational design of the hybrids herein reported.

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Peptide- and NMR-based screening assay for inhibitor of protein-protein interactions

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Protein–protein interactions (PPIs) are key elements of several important biological processes and have emerged as valuable targets in medicinal chemistry. Interactions between proteins are involved in the control of nearly all cellular functions. The network of binary protein-protein interactions (PPIs), the so-called interactome, is extremely expanded, and over 14,000 PPIs have been characterized in humans to date (1). The highly important role of PPIs in living organisms contributes to various pathological states, which has been demonstrated for numerous PPIs associated with the development of human diseases, especially cancer (2). As valuable medicinal chemistry molecular targets, PPIs have gained tremendous attention and substantial efforts have been undertaken to identify effective PPI inhibitors (3). Proteins typically interact via large surfaces, although it is possible to indicate 'hot spots' that are crucial for these processes in many cases. Interestingly, PPIs are frequently dominated by a continuous binding epitope (hot segment) and it is the presence of a dominant hot segment at a protein-protein interface that often renders this PPI druggable (4).
Isolated peptides encompassing hot segment (hot-peptides) often maintain the capability to bind the counterpart protein with different degrees of stability. Here we describe a convenient method for screening of putative PPI inhibitors based on the use of short peptides and ligand-based NMR techniques. The method will be applied to the p53-HDM2 interaction as a case study.

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Lead development of thiazolylsulfonamides with Carbonic Anhydrase Inhibitory action

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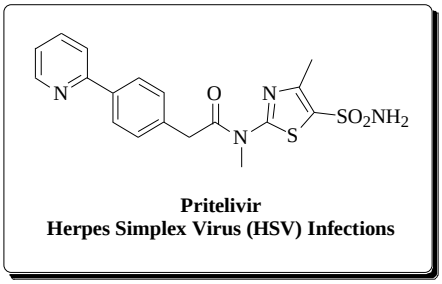
A series of congeners structurally related to pritelivir, *N*-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-*N*-methyl-2-[4-(2-pyridinyl)phenyl]acetamide, a helicase-primase inhibitor for the treatment of herpes simplex virus infections, was prepared.
The synthesized primary and secondary sulfonamides were investigated as inhibitors of six physiologically and pharmacologically relevant human (h) carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, the cytosolic enzymes hCA I and II, the mitochondrial ones hCA VA and VB, and the transmembrane, tumor associated hCA IX and XII.
Low nanomolar inhibition K_I values were detected for all of them, with a very interesting and well-defined structure–activity relationship. As many CAs are involved in serious pathologies, among which are cancer, obesity, epilepsy, glaucoma, etc., sulfonamide inhibitors as those reported here may be of interest as drug candidates. Furthermore, pritelivir itself is an effective inhibitor of some CAs, also inhibiting whole blood enzymes from several mammalian species, which may be a favorable pharmacokinetic feature of the drug which can be transported throughout the body bound to blood CA I and II. (1)



Pritelivir
Herpes Simplex Virus (HSV) Infections

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Predicting CDC25 inhibitors with machine learning approaches

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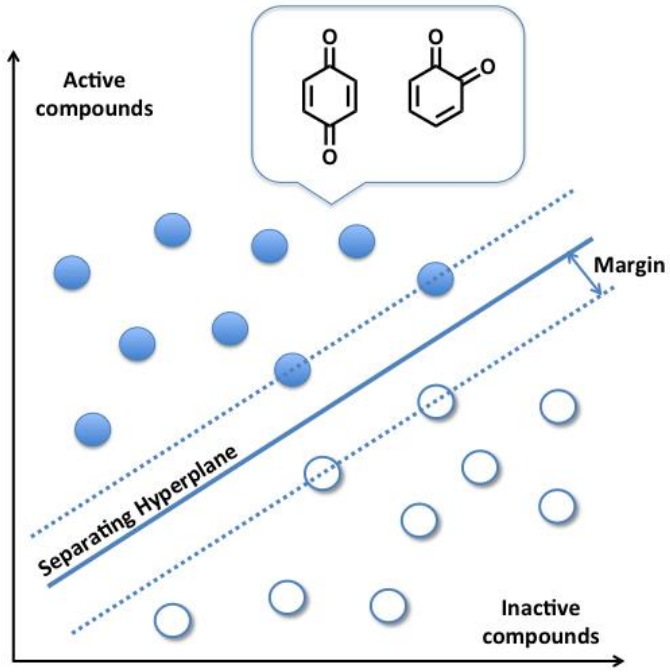
Cell division cycle 25 proteins (CDC25s) are dual-specificity phosphatases acting as key regulators of the cell cycle. CDC25s overexpression has been reported in a significant number of human cancers and has been associated with a poor clinical prognosis. (1) Therefore, CDC25s represent promising targets for the development of anti-cancer drugs. Most of the CDC25 inhibitors reported so far are phosphate surrogates, electrophilic entities and quinonoid compounds that are likely to act through irreversible oxidation of the catalytic cysteine residue. (2,3,4,5) Thus, discerning new chemotypes remains highly desirable. Here we report our strategy to predict CDC25B inhibitors with Support Vector Machine (SVM), one of the most widely used supervised machine learning methods because of the high predictive performance in compound classification and virtual screening. (6,7) A set of CDC25B inhibitors, representing the positive instances, was extracted from ChEMBL by applying stringent selection criteria, in order to obtain only high confidence data. As negative instances, compounds were randomly selected from ZINC. The influence of varying the number of quinonoid compounds in the training set on the model performance was also investigated. The obtained model can be applied to predict new scaffolds and inspire new CDC25 inhibitors design.



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Peptidomics investigation of *Spirulina platensis* after simulated gastro-intestinal digestion by Ultra High Pressure Liquid Chromatography-High resolution Mass spectrometry (UHPLC-HRMS)

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Microalgae are a rich source of bioactive compounds such as proteins, peptides, carotenoids, polyphenols, polyunsaturated fatty acids and more. Among these Spirulina possesses healthy properties. Its major proteins, phycobiliprotein, has several therapeutic activities, namely, hepatoprotective, anti-inflammatory, immunomodulating, antioxidant and anticancer effects (1). With the aim to investigate the release of bioactive peptides, an *in vitro* simulated gastro-intestinal digestion has been carried out on the protein extract of *Spirulina platensis*. The protein fraction was obtained by thermal shock cycles and subjected to digestion protocol. Crude digest was purified and concentrated by Solid Phase Extraction (SPE) by employing Reversed Phase polymeric sorbents. Subsequently, the digest was subjected to UHPLC-HRMS analysis. Peptides were separated on a superficially porous C18 column (100 × 2.1 mm, 1.7 μm) and identified by both Orbitrap and Ion trap-Time of Flight mass spectrometry with the support of Bioinformatics tools. The research led to the identification in the digest of 102 peptides derived from Phycocyanin (alpha and beta-subunits) and Allophycocyanin (alpha and beta- subunits). Peptide extracts were tested ex-vivo on rat blood vessels, showing promising antihypertensive activity. Moreover, the most abundant peptides were synthetized by F-MOC chemistry and tested for further biological evaluation. These data evidence the high nutraceutical value of Spirulina peptides.

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Adsorption of metal ions from a nutraceutically relevant (Poly)phenol aqueous solution by Calcium Carbonate nanoparticles

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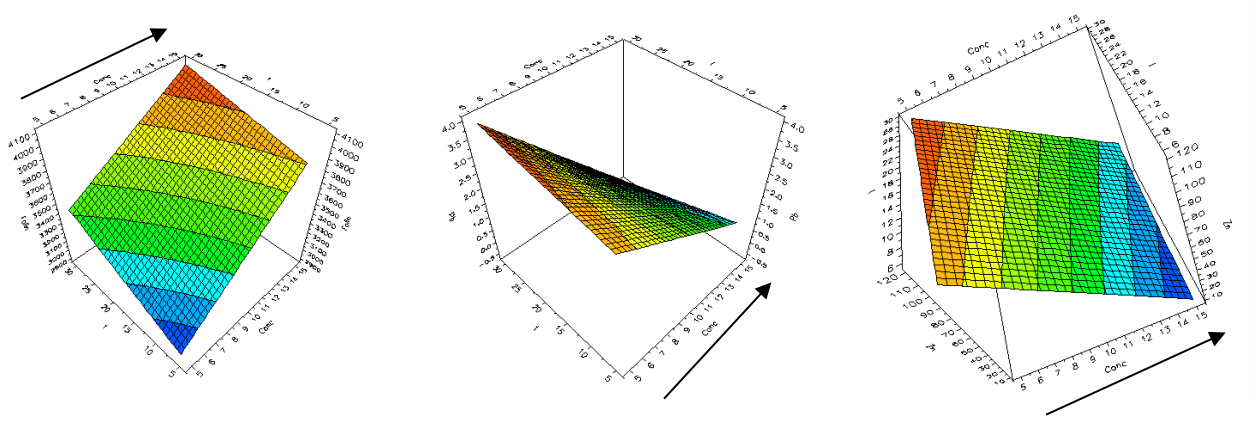
The presence of relatively high concentrations of metals, although still within the limits imposed by law, can reduce the nutraceutical value of (poly)phenolic complexes of plant derivation. Reducing metal concentrations could facilitate preclinical evaluation and would allow better clinical outcomes. This communication will illustrate the possibility of reducing the concentration of metals in a highly concentrated (poly)phenolic aqueous solution obtained by extraction from the juice of Apulian olives. Attempts have been made using methods compatible with the intended use of (poly)phenol complexes as nutraceuticals. Our goal was to reduce the metal ion concentrations without affecting the (poly)phenolic content. The results obtained by treating the aqueous solution with calcium carbonate nanoparticles will be reported. The optimization of the experimental parameters was obtained through the Design of Experiments (DoE) approach (1). The chemiometric model indicated that the best results in terms of (poly)phenoyl residues (Folin; A) and metal abatement (e. g., zinc and lead, ICP-MS, B and C) are obtained with relatively high amounts of nanoparticles. The results can be rationalized by admitting that the metal abatement process frees amounts of (poly)phenols otherwise complexed with metal cations.



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Molecular optimization of *O*-glycoside inhibitors of blood coagulation factors

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Dabigatran etexilate, a selective thrombin (fIIa) inhibitor, and factor Xa (fXa)-selective inhibitors, namely apixaban and rivaroxaban, are new oral active anticoagulants (NOACs), which overcome a number of drawbacks associated to traditional oral anticoagulants (e.g., warfarin) in the therapy of thrombotic disorders (1). Recently, we reported compound **1**, an isonipecotamide-based inhibitor of the serine proteases of the blood coagulation cascade (2,3,4), and its β-D-glucose-containing analogue **2**. The latter compound proved to be a picomolar inhibitor of fXa, with good anticoagulant and profibrinolytic activities. Interestingly, glucosilation resulted in a significant increase of fXa/fIIa inhibition (2,4) (**2**, fXa K_i = 0.090 nM; fIIa K_i = 100 nM). As shown previously, the chlorothiophene moiety is essential for binding of both compounds, whereas comparing the inhibition constant value of **2** with that of the parent compound **1** clearly showed that the removal of the glucose moiety reduces the affinity for fXa by less than ten-fold and for fIIa by more than two orders of magnitude. Moreover, removing the piperidine moiety does decrease affinity to fXa by several orders of magnitude.
Experimental deconstruction of **2** into smaller fragments revealed a binding cooperativity of the piperidine and propylene-linked β-D-glucose fragments, stronger in fIIa (15.5 kJ·mol^{-1}) than in fXa (2.8 kJ·mol^{-1}). For a better understanding of the observed binding cooperativity, the crystal structure of the human α-thrombin in complex with the *O*-glucoside derivative **2** (pdb: 4N3L) has been determined at 1.94 Å resolution, which revealed critical hydrogen bond interactions between the glucose moiety and two basic residues of the Na^+-binding site (R221a and K224), involved in allosteric activation of thrombin.

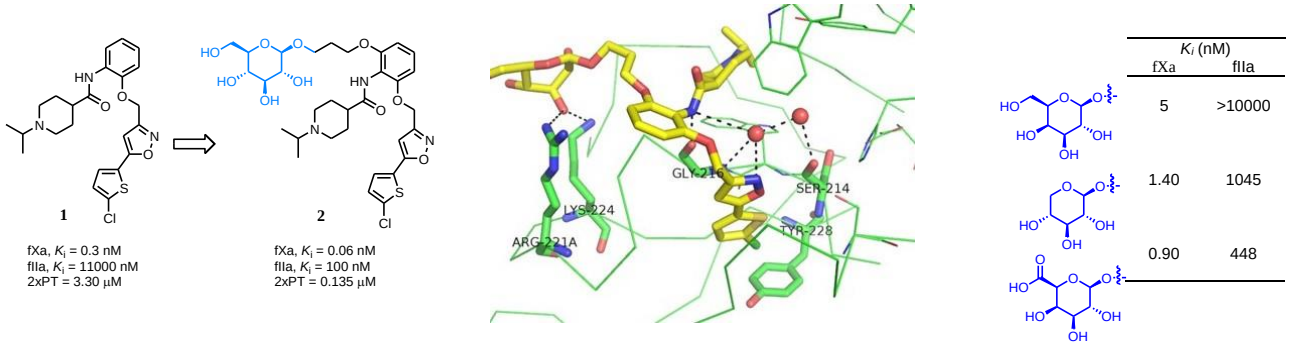

Figure 1. Binding mode of **2** to human α-thrombin as revealed by X-ray crystallography. The ligand structure and the interacting protein residues are shown as stick representation with O in red, N in blue, S in dark yellow, and Cl in dark green; C atoms are in yellow and green for ligand and protein residues, respectively. Structures and bioactivity data of compounds **1** and **2** and their glycoside analogues.

Replacing the glucose moieties with other sugars (i.e. galactose, xylose, and glucuronic acid) revealed the importance of maintaining the β-glucose moiety to stabilize the ligand/enzymes complex. Surface plasmon resonance (SPR) studies and docking calculations provided helpful information for optimizing the design of novel fXa/fIIa inhibitors. SPR has been also used to preliminarily assess parameters related to bioavailability.

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Design of coumarin-based Carbonic Anhydrase IX inhibitors from a fragment pharmacophore model approach

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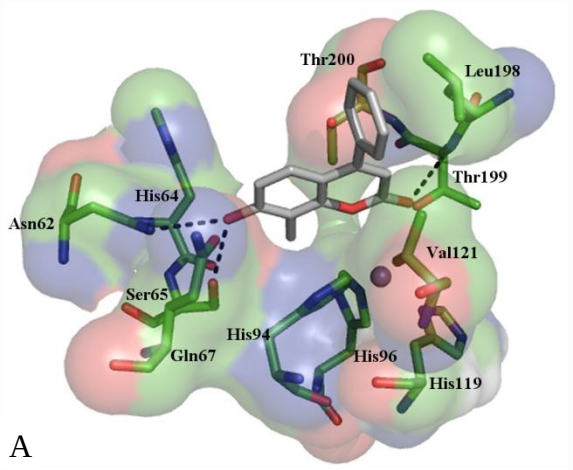
Carbonic anhydrases (CAs) are metalloenzymes catalysing the hydration of carbon dioxide into bicarbonate and proton. There are 15 different human α-carbonic anhydrase isoforms (hCA), which differ from catalytic activity, sub-cellular localization and organ/tissue distribution. The hCAs have basic physiological roles such as breathing, acid-base balance, calcification, secretion of electrolytes and biosynthetic reactions. It is well-known that several isoforms (hCA IX, hCA XII and hCA XIV) are involved in oncogenesis and tumor progression, thus representing molecular targets for the development of anticancer agents (1). Recently, we have identified a new series of coumarin derivatives acting as selective inhibitors of hCA IX over ubiquitous hCA II isoform. A promising compound is the 7-hydroxy-8-methyl-4-phenyl-3,4-dihydro-2H-1-benzopyran-2-one (**1**, K_i = 39.5 nM), for which the plausible binding mode into the catalytic site of hCA IX has been obtained by docking studies performed using AutoDock program (figure A). These results prompted us to exploit this class of selective inhibitors and design new analogues.



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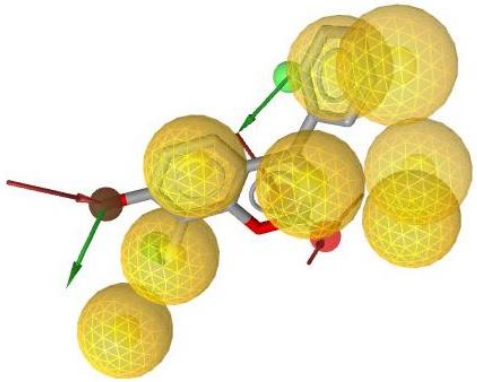

B

Firstly, the main interactions between hCA IX and coumarin **1** have been used as basic information to construct the receptor-based pharmacophore model by LigandScout software. Then, some features have been added as result of an in-depth study concerning the regions surrounding the active site of the apo protein. Therefore, a comprehensive pharmacophore map was obtained (figure B) and it was split in two different fragment pharmacophore models.
Thus, a fragment virtual screening has been performed, based on the versatile and easy synthetic procedure employed to obtain **1**. So further coumarin derivatives were constructed making a selection on the basis of the docking pose and pharmacophore fit value. The selected compounds were synthesized and screened as CA inhibitors.

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In situ gelling Ac2-26 loaded submicrometric particles as wound healing drug delivery systems

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Wound healing is a dynamic ordered process involving a variety of cellular and matrix components that, in some cases, fails in various pathological conditions. ANXA1 has been involved in a broad range of molecular and cellular processes, and its N-terminal derived peptide Ac2-26 is able to activate all three human formyl peptide receptors, promoting calcium fluxes and cell migration, stimulating healing process (1,2). A number of wound dressing devices loaded with active pharmaceutical ingredients have been developed using different polymeric materials. In situ forming gels may combine most of the required properties for an ideal topical formulation (good exudate absorbance, good adherence and removal) with powder easy administration (3). In the present study, we investigated the feasibility of using nanospray drying technology to produce Ac2-26 loaded submicrometric particles able to gel in situ when in contact with wound exudates. Particles have been manufactured using high mannuronic alginate (A), amidated low methoxyl pectin (P) and low molecular weight chitosan (C) for local controlled drug release formulation with enhanced wound healing activity. All formulations loaded with different amount of AC2-26 peptide presented a mean diameter around 750 nm and were able to stabilize the peptide for more than 3 months even at room temperature, where the pure peptide in solid form rapidly degrade after one week. Moreover, the powder was able to move rapidly into a gel when in contact with wound fluids (3-5 minutes) depending on alginate concentration. Proper adhesiveness to of the gel at wound site was found for the most concentrated alginate formulation. Besides, values of all formulations were in a range for easily removal of the formulation after use. Moisture transmission of the in situ formed hydrogel was between 95 and 90 g/m^2/h, an optimum range to avoid wound dehydration or occlusion phenomena (3). Release behaviour of Ac2-26 was directly correlated to peptide and polymeric concentration, resulting in positive burst effect in the first hours of administration followed by a prolonged release till 7 days for the most effective formulations.

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Antifungal extracts from Chestnut (*Castanea sativa*) by-products: characterization and *in vitro* activity against phytopathogenic fungi

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The need to replace synthetic fungicides used against phytopathogenic fungi, whose security has been questioned, has promoted the research on new sources of active compounds. Phytopathogenic fungi are very detrimental for fruit and vegetable productive systems, causing both yield losses and food decay also determining serious risks for consumers, due to the production of dangerous secondary metabolites [1]. Widespread use of synthetic fungicides involves the development of resistant strains, and raises environmental and human health concerns. Natural plant extracts and derivatives, harvesting and shelling two waste products are produced, the bur and the shell, the latter it is studied as antioxidant characterized by a good toxicological and ecotoxicological profile, and with antimicrobial properties may represent an attractive alternative [2]. As a result of *Castanea sativa* source and currently used as fuel. The disposal of these waste materials represents a serious environmental problem, consequently their recovery and recycling may be of a great economic interest. Our investigation has been directed to burs representing a significant by-product of the edible chestnut productive chain and a potential inexpensive source of active phenolics, with antioxidant properties useful in pharmaceutical, cosmetic or food packaging applications [3]. In the present research, the efficacy of methanolic, hydrohalcoholic and aqueous (decoction) extracts from burs against *Alternaria alternata*, *Fusarium solani*, and *Botrytis cinerea* was investigated [2]. Mycelial growth and spore germination rates of the fungi were significantly reduced *in vitro* under exposure to all *C. sativa* bur extracts in a dose-dependent manner. The water-soluble fraction of the methanolic extract showed the highest inhibitory effect. Its main components were isolated and their chemical structures characterized by NMR and MS. Phenolic acids, several flavonol glycosides (kaempferol and quercetin derivatives), phenol glucoside gallates (cretanin, chesnatin, chestanin) and C-glycosil ellagitannins (castacrenin A and B) were detected. The marker compounds were identified as quercetin 3-O-β-D-glucopyranoside and chestanin, and their quantitative analysis was performed by HPLC-DAD. Results suggested that the major antifungal efficacy of this fraction is due to both higher total phenol (as determined by Folin Ciocalteu test) and markers content. Its radical scavenging activity (against DPPH and ABTS radicals) was higher than hydrohalcolic and aqueous extracts. Our results showed that chestnut wastes have promising prospects for the utilisation to reduce the using of antifungal chemicals and to achieve a more sustainable use of pesticides.

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The role of Transcutol® on skin penetration ability of diclofenac acid nanosuspensions

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The poor ability of many drugs to permeate the skin layers is the main limiting factor for the exploitation of the transdermal route for drug delivery. As a consequence, several approaches have been proposed to overcome the skin barrier, such as the inclusion of penetration enhancers in the topically applied drug formulations. Another novel approach to increase skin permeability of poorly water soluble drugs is the production of nanocrystals (pure drug crystals with an average diameter below 1 µm stabilized with a small amount of stabilizer) (1).

In this work novel diclofenac acid nanocrystal formulations were developed using the wet media milling technique, Poloxamer 188 as stabilizer and the penetration enhancer Transcutol® (diethylene glycol monoethyl ether) as excipient (2).

Formulations were characterized by different techniques such as scanning electron microscopy, differential scanning calorimetry, X-ray powder diffractometry, Fourier-transform infrared spectroscopy and photon correlation spectroscopy. The influence of diethylene glycol monoethyl ether on (trans)dermal delivery of diclofenac topically applied as nanosuspensions was evaluated by *in vitro* studies using Franz diffusion cells and pig skin. Diclofenac nanosuspensions without the penetration enhancer, diclofenac coarse suspensions and a commercial gel containing diclofenac sodium were used as controls.

Results demonstrated that the presence of diethylene glycol monoethyl ether influences the Poloxamer 188 ability to stabilize the nanocrystals during the milling process. Indeed, nanosuspensions with the penetration enhancer exhibited a mean diameter greater than those of the nanosuspension without it. Moreover, in vitro permeation studies showed that the nanosuspension without diethylene glycol monoethyl ether enhanced diclofenac acid skin delivery compared to coarse suspension and the commercial gel, thus indicating that the nanosizing process and the different ability of diclofenac sodium salt and diclofenac acid to permeate into the skin play a key role in the dermal penetration process. Finally, increased concentrations of the penetration enhancer decreased the diclofenac acid skin accumulation in the stratum corneum.

Overall, the present results exclude a synergistic effect of the nanosizing approach and the addition of diethylene glycol monoethyl ether on the skin penetration of diclofenac applied as a nanosuspension.

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Topically applied baicalin gellan-transfersomes: *in vitro* and *in vivo* evaluation

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In the present work, nanotechnologies of gellan-nanohydrogel and phospholipid vesicles were combined to incorporate baicalin in new gellan-transfersomes obtained by an easy and scalable method. Specifically, the polyphenol was incorporated in transfersomes prepared with soy phosphatidylcholine(Lipoid S75) and tween 80(1,2). Then, considering advantages of the association of phospholipid vesicles and hydrogels, as improvement achieved in skin delivery and formulation stability, transfersomes were combined with a gellan-cholesterol derivative, which is expected to stably interact with the vesicle bilayer due to its amphipathic nature stemming from the hydrophilic polymeric chains and the lipophilic cholesterol(3).Moreover, this combination may improve both vesicle viscosity and skin delivery capabilities.Nanohydrogels of gellan-cholesterol derivative were produced by ultrasound or autoclave treatment of the polymer suspension, and then used as hydrating medium for the preparation of two different baicalin loaded gellan-transfersomes.Empty and baicalin loaded transfersomes were small in size (~80 nm) and monodispersed (PI ~0.19). The use of the gellan-nanohydrogels as hydrating medium led to the formation of larger vesicles, especially baicalin loaded sonicated gellan-transfersomes, with a mean diameter ~123 nm. The zeta potential was similar for all the nanovesicles, ~-50 mV, due to the contribution of negatively charged S75. Cryo-TEM showed the actual formation of lamellar vesicles in all the three samples. In particular, transfersomes were spherical and unilamellar,sonicated gellan-transfersomes were unilamellar with a peculiar oval and elongated rod-like shape and autoclaved gellan-transfersomes were unilamellar, with irregular round shape.The entrapment efficiency was ~37% for transfersomes and ~45% for gellan-transfersomes, thus,suggesting that baicalin is loaded within the vesicles, but also embedded in the three-dimensional network of the gellan-cholesterol chains, as previously reported for other tree-dimensional vesicle dispersions (4).Gellan was anchored to the bilayer domains through cholesterol, and the polymer chains were distributed onto the outer surface of the bilayer, thus, forming a core-shell structure, as suggested by rheological studies and SAXS analyses. The optimal carrier ability of core-shell gellan-transfersomes was established by the enhanced skin deposition of baicalin, especially in the deeper tissues.Core-shell gellan-transfersomes, especially the system based on autoclaved gellan-nanohydrogel, provided the greatest baicalin *in vitro* deposition in intact skin, thanks to the peculiar assembling structure where the external gellan chains, surrounding the vesicles, favored their adhesion to the skin surface and promotedvesicle diffusion. Moreover, their ability to improve baicalin efficacy in anti-inflammatory and skin repair tests was confirmed *in vivo* in mice, providing the complete skin restoration and inhibiting all the studied inflammatory markers (oedema, MPOand TNFα).

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Discovery of store-operated Calcium entry modulators as an effective treatment for calcium-related rare genetic diseases

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Store Operated Calcium Entry (SOCE) is the major route of replenishment of intracellular Ca^{2+} in response to depletion of Ca^{2+} stores in the endoplasmic reticulum (ER). The key molecular components of SOCE machinery are STIM proteins, which function as endoplasmic reticulum calcium sensor, and Orai channels.(1)

Recently, several human diseases have been associated with mutations in these two proteins: loss-of-function mutations result in SCID-like immunodeficiencies, while gain-of-function mutations cause Stormorken syndrome, York platelet syndrome and tubular aggregate myopathy (TAM).(2) These pathologies are rare diseases with an estimated prevalence of 1 every 500 births and are currently without therapy.

Due to the recent discovery of STIM and Orai proteins, structural information is poor and only a low resolution crystal structure of Orai from *Drosophila melanogaster* has been described.(3) Therefore, the search for SOCE modulators perfectly suited to a click chemistry approach. Starting from the structure of known pyrazole derivatives (BTP, Pyr),(4) a library of candidates was designed and synthesized. Screening was performed by calcium microfluorography in wild type and mutated human embryonic kidney (HEK-293T) cells and led to the identification of both SOCE activators and inhibitors (Figure 1). Selected compounds were further evaluated by electrophysiological experiments and by *ex vivo* studies on muscle biopsies from patients affected by TAM.(5)

Chemical synthesis, metabolic stability profile and biological evaluation of this class of compounds will be discussed.

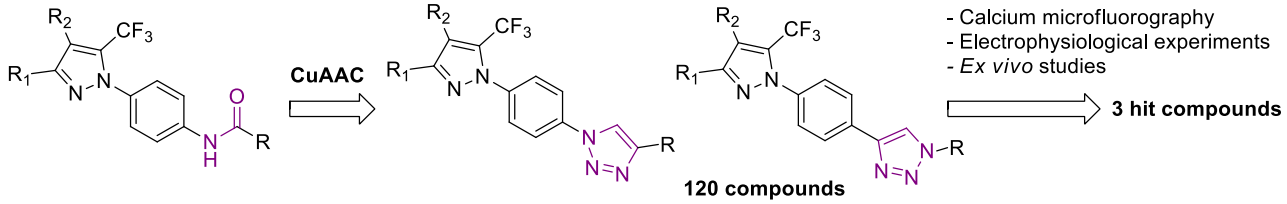

Figure 1

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Computational and experimental structural studies leading to new potent Tyrosinase inhibitors bearing 4-Fluorobenzyl moiety

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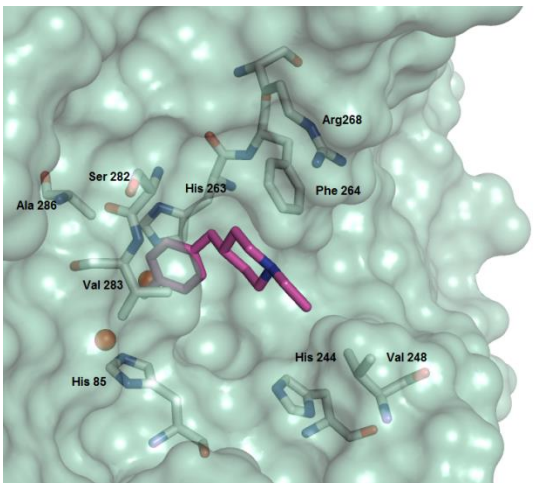
Tyrosinase (TY) is a copper-containing glycoprotein widely distributed in nature and belonging to the type 3 of copper protein family. TY catalyses and plays a key role in melanin biosynthetic pathway. Although the melanin production shields the human skin from UV radiation, inhibiting photocarcinogenesis and affecting the synthesis of vitamin D3, an excessive accumulation, or an irregular distribution, can lead to serious cutaneous pigmentation disorders (1). Thus, in the last few years many efforts have been made to identify new and potent enzymatic inhibitors useful in clinical therapeutic applications as well as in cosmetic industry. Recently, we reported small synthetic molecules as a new class of TY inhibitors and some of them displayed higher efficacy than the well-known reference compound kojic acid.

Specifically, the most active inhibitor 1-(5,6-dimethoxy-1*H*-indol-3-yl)-2-(4-(4-fluorobenzyl)piperidin-1-yl)propan-1-one showed promising IC_{50} value of 7.56 μM and affected diphenolase activity as mixed-inhibitor (2). The structure activity relationship considerations suggested that 4'-fluorobenzyl moiety could exert a crucial role in controlling inhibitory effects. Therefore, we have explored the docking poses of a new series of compounds able to set the 4-fluorobenzyl fragment in the hole of catalytic site.



Then selected compounds were synthesised and assayed against TY, thus identifying new potent inhibitors ($IC_{50} \leq 2.03$ μM) when compared with kojic acid (IC_{50}=17.76 μM). Notably, the co-crystal structure with TY confirmed that the 4-fluorobenzyl moiety is situated between the two copper ions, with the aromatic ring stabilized through stacking interactions within hydrophobic wall of catalytic pocket.

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Synthesis of nabumetone analogues for topical use: photodegradation studies and design of light-stable formulations

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Photostability studies applied on topical formulations containing anti-inflammatory drugs have confirmed the sensitivity to light of many of these drugs (1,2). For this reason, their formulation in cream or gel is often avoided in favor of other forms, such as tablets or suspensions.
In this work, the behavior of nabumetone (NA), (4- (6-methoxy-2-naphthyl) butan-2-one) in aqueous solution was tested, revealing the 6-methoxy-naphthalene-aldehyde as the main photoproduct (3). Photodegradation of NA was then investigated in both liquid and gel formulations, according to the ICH rules (4). The experiments were monitored by spectrophotometry and the data processed by Multivariate Curve Resolution (MCR), able to estimate spectra and concentration profiles of the components involved in the kinetic process.
Photostabilization of the drug is proposed by two different approaches:

1. Design and development of specific NA analogs with greater stability and fewer side effects.
2. Incorporation in cyclodextrin matrices aiming to improve the light-stability of NA and analogues in topical formulations.

The new synthetized compounds were designed on the base of the receptor binding-site features, by computer-aided approach. In particular, the compounds with a lactone moiety mimicking the linear butan-2-one portion of NA were prepared. The synthesis of the designed compounds was achieved by newly synthetic strategies as well as optimization of previously reported procedures, with the aim of obtaining compounds with high yield, purity and stability.
All the compounds were incorporated in cyclodextrin matrices and the complexes exposed to forced degradation to test their ability in improving the light-stability. Several type of cyclodextrins were evaluated to increase the encapsulation percentage of the drugs.

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Design and synthesis of novel macrocyclic Melanocortin peptides: discovery of potent and selective ligands at hMC3 and hMC5 receptors

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The melanocortin system includes five receptor isoforms known as MC1R-MC5R, which are involved in a large variety of physiological functions and are distributed in several different tissues (1). The endogenous ligands, the melanotropins, which bind to these receptors are linear peptides, α-, β-, γ-MSH, and ACTH, and are endowed of low selectivity and therefore the physiological function of each receptor subtype can't be easily delineated. Thus, there is an urgent need for the synthesis of ligands highly selective which would be useful pharmacological tools for further receptor investigation (2,3).

To date, only few synthetic ligands active at hMC1 and hMC5 receptors are available but most do not have appreciable selectivity. Thus, with the aim to discover new potent and selective ligands we designed novel macrocyclic compounds in which a constrained amino acid residue was inserted between His^6 and Trp^9 by a lactam bridge using a Glu or Asp residue. We designed and synthesized 2 series of macrocyclic compounds containing Glu or Asp, respectively. The resulting macrocyclic peptidomimetics, characterized to have a 19 or 20-membered ring, conserved the melanocortin core sequence His-Phe/Nal(2')-Arg-Trp (Figure 1).

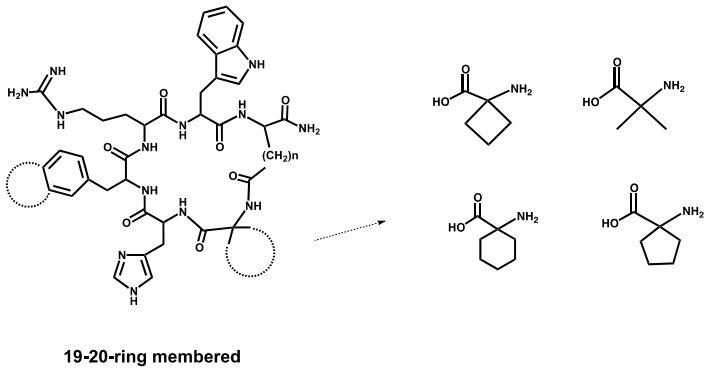

Figure 1. Macrocyclic compounds mimics of melanocortin peptides.

The main intent of the current study was to examine this kind of macrocyclization as an additional approach toward development of MT-II/SHU9119 analogues with enhanced receptor selectivity (4). All synthesized compounds were evaluated for their binding affinities at the human melanocortin receptors 1-5 in competitive binding assays using the radiolabeled ligand [^{125}I]-NDP-α-MSH, and for their agonist potency in cAMP assays employing the HEK293 cells expressing those receptors.

Here, we report the biological activity and the preliminary conformation properties of synthesized compounds.

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Design, synthesis and spectroscopic evaluation of novel fluorescent styryl pyridinium Carbonic Anhydrase inhibitors

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Fluorescence emission by organic molecules is a phenomenon strictly dependent on the surrounding microenvironment. (1) The formation of discrete host/guest complexes of fluorescent dyes with macrocyclic structures has been widely documented (1) and was generally found to elicit a consistent change in the micro-environmental parameters, which subsequently perturbs the fluorescence phenomenon. Fluorescent dyes host/guest complexes possess potential biological and environmental applications in the areas of sensing and signaling.(1) Hence, we designed a set of 4-[4-(dimethylamino)styryl]pyridium based fluorescent dyes (2) bearing classical zinc binding groups (ZBG) such as the sulfonamides, sulfamates and sulfamides to address their spectrum of action to the inhibition of the Zn enzymes carbonic anhydrases (CAs, EC 4.2.1.1). (3,4) The reported derivatives were evaluated for their inhibition profiles against four physiologically relevant human (h) CAs, isoforms hCA I, II, IV and XII. The synthesized dyes demonstrated to possess diverse inhibitory potency depending on the nature of the exhibited ZBG and on the length of the spacer between the fluorescent core and the ZBG itself. The formation of supramolecular host/guest biological complexes was reported by means of UV-vis absorption and fluorescence emission measurements, which were carried out for all the reported derivatives alone and in presence of the ubiquitous isoforms hCA I and hCA II. The X-ray crystal structures of four of the aforementioned host-guest CA-inhibitors complexes were obtained and provided for a valid explanation for the spectroscopic changes the dyes revealed after incubation with the two enzymatic isoforms.

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First profiling of flavonoids in Tarocco "Lempso" (*Citrus Sinensis* L. Osbeck) clone variety and its antioxidant potential by DPPH-UHPLC-PDA-IT-TOF

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Clonal selection and hybridization are valid strategies to obtain fruits with enhanced sensorial and nutraceutical properties (1,2). Within *Citrus sinensis* varieties, Tarocco clone "Lempso" is a typical product of Calabria region (Italy) characterized from a red pulp. This is the first report concerning its accurate profiling.

To characterize in detail the flavonoid composition of Lempso clone and to compare its antioxidant potential with other Citrus varieties by a fast screening method, extracts were subjected to solid phase extraction and the quali/quantitative profile was elucidated through ultra high performance liquid chromatography (UHPLC) coupled to photodiode array (PDA) and ion trap-time of flight (IT-TOF) mass spectrometry detection, and compared to both Cleopatra mandarin (*Citrus reticulata*) and blood orange (*Citrus sinensis* (L.) Osbeck) Sanguinello varieties. The antioxidant activity was assessed by pre-column DPPH reaction coupled to UHPLC-PDA (3).

Lempso is characterized by flavonoids and anthocyanins. Flavanones content (Hesperidin: 57.19 ± 0.49, Vicenin-2: 4.59 ± 0.03, Narirutin: 5.78 ± 0.13 mg/100 mL) was considerably higher than Cleopatra and Sanguinello varieties. The developed DPPH-UHPLC-PDA method provides information regarding the single contributions to antioxidant activity, highlighting how Ferulic acid, Quercetin and Cyanidin derivatives possess considerable radical scavenging activity (> 50%) (4,5). The total antioxidant activity was also evaluated and compared with positive controls, showing higher scavenging activity than Cleopatra and Sanguinello (IC50: 333.76 ± 10.81 μg/mL vs 452.62 ± 10.81 and 568.39 ± 26.98 μg/mL, respectively).

These data evidence the nutraceutical potential of Lempso variety, which could be an ingredient for functional beverages.

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Benzofuran derivatives: a new class of 'direct' AMPK activators

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AMPK (adenosine monophosphate-activated protein kinase) is a serine/threonine heterotrimeric kinase comprising a catalytic subunit (α) and two regulatory subunits (β and γ). It is significantly involved in the regulation of energy demanding/consuming metabolic pathways, playing a key role in maintaining suitable ATP cell levels under conditions depleting energy levels such as exercise, starvation, hypoxia and rapid cell growth. Thanks to the central role played by AMPK in cellular and whole body energy homeostasis, this protein represents an attractive target for the treatment of a number of metabolic diseases, including type 2 diabetes and obesity, as well as of immune-mediated inflammatory diseases and cancer, thus highlighting the persistent need for effective and potent activators (1). Different classes of AMPK activators have been developed, the main relevant one being represented by the so called 'direct' activators (2).

We developed a novel class of 'direct' AMPK activators, which target the AMP binding site located at the AMPK-γ regulatory subunit of the protein (Fig. 1a) (3). The novel derivatives, characterized by a 3-amino-5(6)-arylbenzofuran-2-carboxamide structure, possess key pharmacophoric elements that allow a profitable interaction with the target enzyme. Actually, both the 2-carboxamide portion and the oxygen atom of the core let the compounds hook the AMPK-γ regulatory subunit through H-bond interactions. Moreover, the wide and aromatic benzofuran core confers liphophilicity, thus assuring a suitable interaction with the liphophilic area of the site and conferring, at the same time, a profitable bioavailability. The novel compounds increased significantly the phosphorylation of AMPK at a concentration of 10 μM, and proved to be more potent than the well-known AMPK activator Berberine (BBR) (Fig. 1b). In addition, as it is known that stimulation of phosphorylated AMPK is potentially related to the increase of Sirt1 activity, the effects of the novel compounds on Sirt1 activation was investigated as well. Results of this test confirmed the efficacy of the benzofuran derivatives, making them even more attractive drug candidates due to their activity on Sirt1.

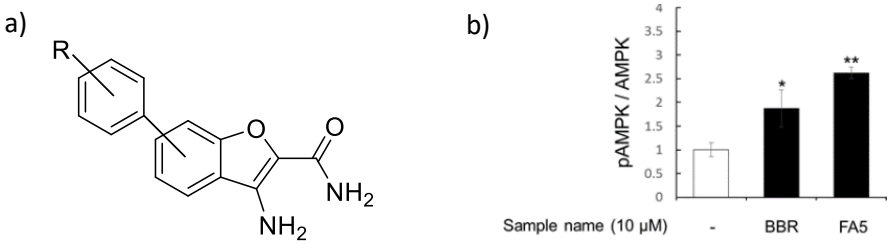

Figure 1

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Structure-activity relationship study of a FHIT-mimetic peptide

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The fragile histidine triad (FHIT) protein is a member of the large and ubiquitous histidine triad (HIT) family of proteins. On the basis of the genetic evidence, it has been postulated that the FHIT protein may function as a tumor suppressor, implying a role for the FHIT protein in carcinogenesis.(1) Recently Gaudio et al. reported that FHIT is in a molecular complex with annexin A4 (ANXA4), following to their binding, FHIT delocalizes ANXA4 from plasma membrane to cytosol in paclitaxel-resistant lung cancer cells, thus restoring their chemosensitivity to the drug.(2) They also identified the smallest region of the FHIT protein sequence still interacting with ANXA4. This short sequence, QHLIKPS, ranging from position 7 to 13 of FHIT protein, was not only able to bind ANXA4 but also to keep it in the cytosol during paclitaxel treatment, thus avoiding ANXA4 translocation to the inner side of cell membrane.(2) Starting from these results, we initiated a systematic SAR study on the peptide mentioned above, through an Ala-scan approach, binding assay and structural studies by CD and NMR.

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Structure-activity relationship studies of lactoferrin-derived peptides active towards influenza virus

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Bovine lactoferrin (bLf) is a multifunctional glycoprotein that plays an important role in innate immunity against infections, including influenza.(1, 2) Therefore, bLf was considered a novel drug target for the inhibition of influenza virus infection. Previously, we have identified three C-lobe bLf-derived tetrapeptides (SKHS, SLDC, VLRP) as the minimum fragments expressing the broad anti-influenza activity of bLf. These tetrapeptides inhibit the Influenza virus hemagglutination and cell infection in a concentration range of femto- to picomolar.
In this study, we performed structure-activity relationship (SAR) studies to generate peptides with improved biological activity. All new derivatives were tested for the assessment of their ability to inhibit viral hemagglutination and cell infection.

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Synthesis, biological evaluation and molecular docking of Ugi and Passerini products as novel indoleamine 2,3-dioxygenase 1 inhibitors

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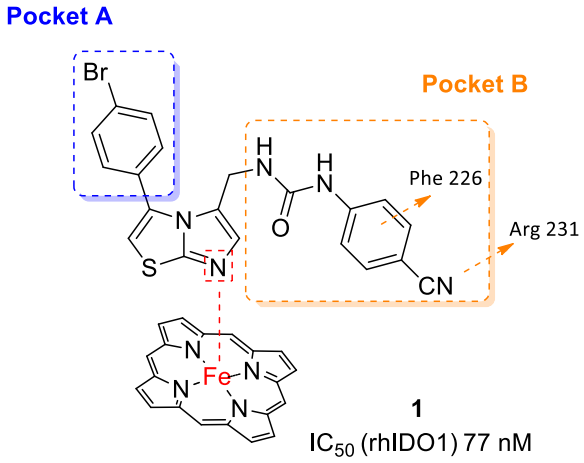
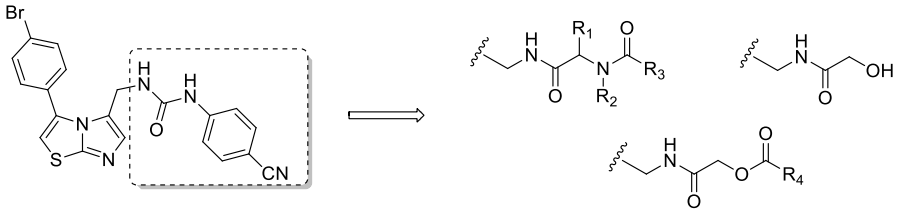
Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme involved in tryptophan catabolism through the kynurenine pathway and plays a central role in pathological immune escape process(1). IDO1, overexpressed in a variety of diseases, including cancer and neurodegenerative disorders(2), is emerging as an attractive target for immunological cancer treatment. Recently, imidazole(3) and imidazothiazole(4) derivatives have been discovered as promising IDO1 inhibitors. Among them, **1** is the most potent compound identified so far (Scheme 1), with an IC_{50} value of 77 nM in the enzymatic assay (rhIDO1).



Scheme 1

With the aim of further improving the biological profile and probing interactions with the aminoacids in the catalytic site, we have exploited the Ugi and Passerini multicomponent reactions(6) to access a library of imidazothiazole derivatives with a diversified side-chain (Scheme 2).



Scheme 2

Preparation, biological evaluation and molecular docking of the synthesized compounds will be discussed.

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Coupling online comprehensive hydrophilic interaction chromatography × reversed-phase ultra-high-pressure liquid chromatography with high resolution mass spectrometry: a powerful platform for complex polyphenolic sample analysis

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Given their complexity, multiclass polyphenolic samples require increased selectivity and resolution to thoroughly characterize their components. For this purpose, in this work we developed an improved online comprehensive two-dimensional liquid chromatography platform coupled to tandem mass spectrometry. A narrowbore hydrophilic interaction chromatography column (150 × 2.0 mm, 3.0 µm, cross-linked diol) was employed in the first dimension, while a reversed-phase column based on monodisperse sub-2 µm fully porous particles (50 × 3.0 mm, 1.9 µm d.p.) with high surface area (410 m^2/g) was employed in the second dimension. The combination of a trapping column modulation interface with the high retentive fully porous monodisperse reversed-phase column in the second dimension resulted in higher peak capacity values (1146 versus 867), increased sensitivity, sharper and more symmetrical peaks in comparison with a conventional loop-based method, with the same analysis time (70 min). The system was challenged against a complex polyphenolic extract of a typical Italian apple cultivar, namely Annurca (1), enabling the simultaneous separation of multiple polyphenolic classes in a single analytical run, including oligomeric procyanidins up to degree of polymerization of 10 (2,3). Hyphenation with an ion trap time-of-flight mass spectrometer led to the tentative identification of 121 analytes, showing how this platform could be a powerful analytical tool for the accurate profiling of complex polyphenolic samples.

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Rational design and function prediction of FPR2 ligands based on docking studies and MD simulations

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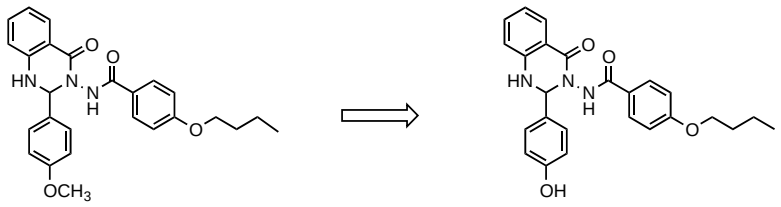
Formyl peptide receptor 2 (FPR2) is a G protein-coupled receptor belonging to the *N*-formyl receptor family (FPRs) (1) that plays critical roles in peripheral and brain inflammatory responses and, as such, it has been considered as an attractive therapeutic target for the development of drugs that could halt pathological inflammatory reactions (2). To date several classes of non peptidic FPR2 agonists have been described, whereas only very few antagonists have been reported. With the aim to identify the molecular determinants responsible for functional properties of FPR2 ligands, we constructed a homology model using two antagonist-bound peptide receptor crystal structures as templates (chemokine CXCR4, and angiotensin AT1R receptor) (3). Docking studies on structurally diverse FPR2 agonists and antagonists were performed (4) using Glide in Schrödinger suite. The poses were clustered and molecular dynamics simulations were conducted for the representative poses using AMBER. For each simulation we monitored nonbonded energy, RMSD and ligand-receptor hydrogen bond formation. We observed that the hydrogen bonds between ligand carbonyl group and Arg201 or Arg205 are generally energetically favored. This interaction, observed for all the investigated ligands, seems an essential feature for FPR2 ligand recognition. Next, we focused on the binding mode of quinazolinone derivatives Quin C1 and Quin C7 (Figure I), in which a simple structural modification interconverted the functional properties from agonism to antagonism. We here present docking studies results and the design of new quinazolinone derivatives.

Figure I



Quin C1
agonist

Quin C7
antagonist

References: 1. R.D. Ye, F. Boulay, J.M. Wang, C. Dahlgren, C. Gerard, M. Parmentier, C.N. Serhan, P.M. Murphy. *Pharmacol. Rev.* 2009, **61**, 119-161. 2. S.N. Cooray, T. Gobbetti, T. Montero-Melendez, S. McArthur, D Thompson, A.J. Clark et al. *Proc Natl Acad Sci U S A.* 2013, **110**, 18232-7. 3. D. Latek, P Pasznik, T Carlomagno, S. Filipek. *PloS one.* 2013, 56742. 4. E. Lacivita, I.A. Schepetkin, M.L. Stama, L.N. Kirpotina, N.A. Colabufo, R. Perrone et al. *Bioorg Med Chem*. 2015, **23**, 3913-24.

Battle against antimicrobial resistance: FtsZ inhibitors as novel potent Gram-positive antibiotics

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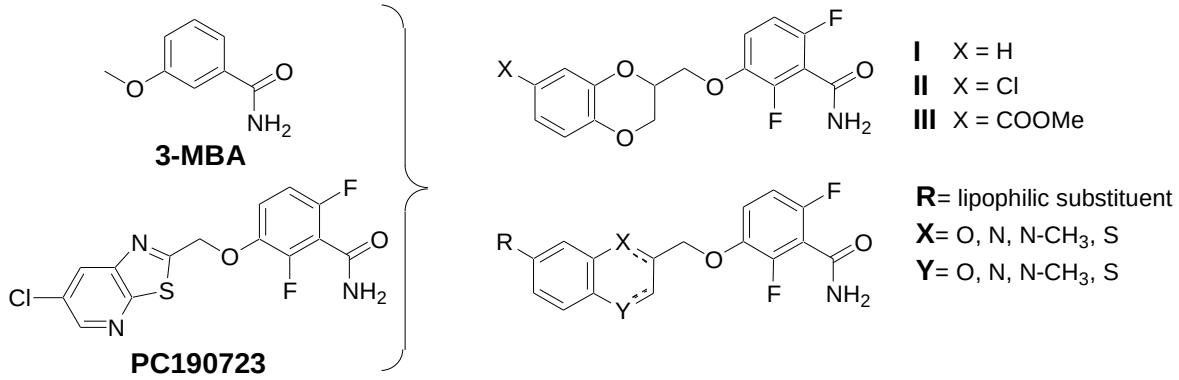
Antimicrobial resistance is one of the major actual health plagues. Even if it started more than 70 years ago, the problem burst out only in the latest years, prompting to the urgent need of novel efficient antibiotics, showing innovative mechanisms of action.
In this context, the bacterial cell division process turned to be an interesting and promising target (1), firstly because divisome components are crucial for the viability of bacteria. Moreover, the most important division proteins are widely conserved in bacteria and are absent in eukaryotic cells, strengthening the selectivity of the possible novel antimicrobics.
Among the essential cell division proteins, FtsZ (Filamentous temperature sensitive Z), which is a tubulin homologue (2), became an attractive target. FtsZ is the first protein that localizes to the mid-point of the cell and it undergoes polymerization in a GTP-dependent manner, bringing to the formation of the Z-ring. It recruits at least ten other cell division proteins, which enable cell constriction, the formation of mesosome and two daughter cells (3).
In the last 10 years several research group studied and developed FtsZ inhibitors, confirming that protein inhibition results in a bactericidal effect. Interesting results were obtained with synthetic small molecules; specifically with 3-Methoxybenzamide (3-MBA) derivatives: the lead compound of this class of antimicrobics is PC190723 (4-6).



In the attempt to design potent novel antibacterial agents, in the latest years we designed and accomplished several derivatives, firstly replacing the thiazolopyridine of PC190723 with differently substituted 1,4-benzodioxane, bringing in particular to compounds I-III (7,8). These molecules proved to be strong inhibitors of *S. aureus, E. faecalis and M. tuberculosis* viability. Recently we consolidated the Structure Activity Relationship (SAR) of this class, designing a number of analogues of I and III, through a series of isosteric, positional or substituent modifications (9).
Furthermore, we confirmed the target, performing two different biochemical assays, aimed at studying GTPase and polymerization activities of S. aureus FtsZ, when incubated with our compounds.

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Novel D- Glucosamine *N*- Peptidyl derivatives endowed with selective activity towards IKK alpha

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Osteoarthritis (OA) is a rheumatic disease which represents the major cause of disability in the adult population as well as a severe health burden with a significant economic impact. OA is the result of abnormal biomechanics and cell-derived and tissue-derived factors. (1) The NF-kB family of nuclear transcription factors is involved in the induction of inflammatory disorders, representing a potential therapeutic target in OA. It comprehends ubiquitously expressed proteins responsible for the regulation of a considerable number of genes. These transcription factors are sequestered in the unstimulated cell cytoplasm by inhibitor proteins called IkBs, forming inactive complexes. As a result of specific stimuli IkB is phosphorylated by IkB kinase (IKK) complex, leading to the dissociation of IkB from NF-kB which can migrate into the nucleus, activating the gene transcription. IKK includes three components: IKKα, IKKβ and NF-Kb essential modulator (NEMO). IKKα and IKKβ are implicated in the regulation of the expression of genes involved in the extracellular matrix remodeling and terminal differentiation of chondrocytes. (2,3) From a random screening of our in house library the compound **RC510** (already known as substrate analog inhibitors of papain and cathepsin-B), (4) a D-glucosamine *N*-peptidyl derivative, showed selective activity towards IKKα. (5) Following this result we decided to investigate the interactions of this compound with the target by conducting molecular docking studies, in order to speculate about the mechanisms by which it binds to IKKα kinase domain. As docking molecular target we used a three-dimensional model of IKKα, built by homology modelling. Docking experiment showed that **RC510** interacts with ATP binding pocket mainly by the establishment of hydrogen bonds (with backbone atoms of Thr15 and Glu140 and with side chains of Thr15 and Asp94) and of hydrophobic interactions. From these results we decided to design and synthesize a novel series of D-glucosamine *N*-peptidyl derivatives in order to obtain compounds having an inhibitory activity towards IKKα.

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Identification of natural products as anti-melanogenesis agents

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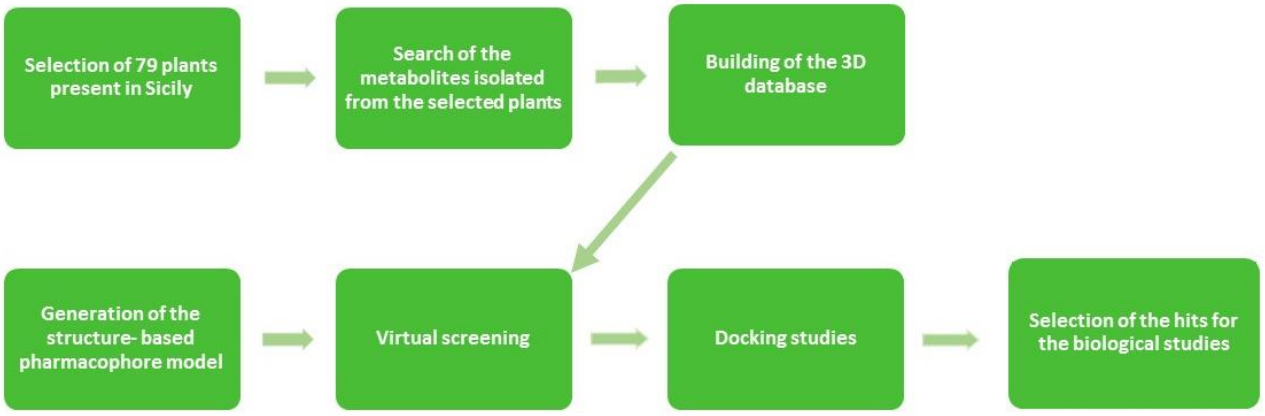
Melanogenesis is a biosynthetic pathway for the formation of melanin pigment in human skin and hair, as well as for the browning of fruit and vegetables. Abnormal production of melanin causes dermatological disorders such as freckles, melasma and cancer. Tyrosinase (EC 1.14.18.1) is the key regulatory enzyme involved in the biosynthesis of melanin pigments. It is a type 3 copper protein widespread in mammals, plants, fungi and bacteria. Specifically this enzyme catalyzes the first two steps of the biosynthetic process: the o-hydroxylation of monophenols and the subsequent oxidation of the resulting o-diphenols into o-quinones. The inhibition of tyrosinase activity represents the most prominent approach to inhibit melanogenesis. A large number of tyrosinase inhibitors have been reported in literature, but their use is limited due to their side effects, low stability and cytotoxicity. This encourages researchers to seek safer tyrosinase inhibitors (1,2).

Herein, structure-based modeling approaches were used to identify new tyrosinase inhibitors from natural sources, considering that the natural products have been and continue to be a rich source for drug discovery. In particular, a pharmacophore model for the tyrosinase enzyme was generated by means of LigandScout software. The obtained model was used to screen the database SiciMet, which has been built in house collecting 791 secondary metabolites from sicilian plants. The hits obtained from the virtual screening runs were subjected to docking studies in order to further investigate both the putative ligand binding-mode within the active site and the biological effects.



References: 1. Chang TS; *Materials*, 2012, *5*, 1661-1685. 2. Bagherzadeh K. et al.; *J Biomol Struct Dy*, 2014, *33(3)*, 487-501.

Polypharmacology predictions in the Protein Data Bank

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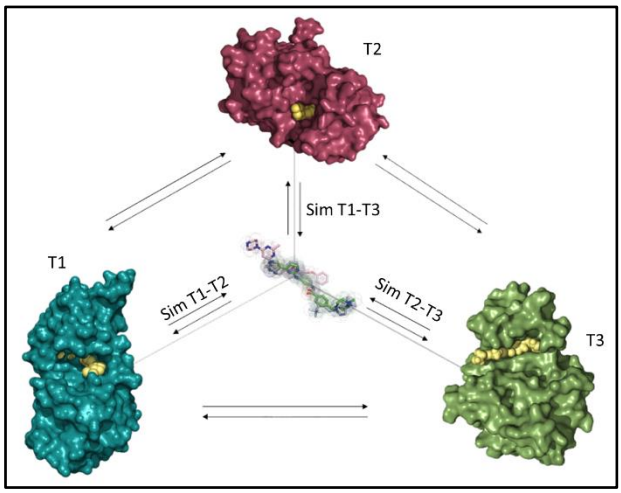
The design of a chemical entity that simultaneously and selectively modulates a selected pool of biological targets represents an attracting goal, especially for the treatment of complex diseases (1). Despite recent successes, two considerations arise: first, *ad hoc* methods to predict the desired polypharmacological profile are needed; second, chemical/structural/biological information contained in publicly available databases is generally not thoroughly exploited to prospectively design polypharmacological compounds (2,3). In this context, the Protein Data Bank (PDB) represents a rich source of information to help predict polypharmacological profiles of ligands. Here, a systematic analysis of the PDB using different integrated computational approaches has been performed. New polypharmacological profiles of ligands deposited into the PDB were established. Moreover, the analysis of the chemical landscape covered by these ligands highlighted interesting relationships between different protein targets and their respective ligands.



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Cavalluzzi Maria Maddalena	FAR PO60
Cavarelli Jean	FAR PO14
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Cerchia Carmen	FAR PO45
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Cerofolini Linda	FAR PO56
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Ciaglia Tania	FAR PO32
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Ciccarella Giovanni	FAR PO60
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Collino Massimo	FAR OR12
Colotti Gianni	FAR OR15
Coluccia Addolorata Maria Luce	FAR PO40
Coluccia Antonio	FAR PO06
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	FAR PO41
Conconi Maria Teresa	FAR PO11
Consalvi Sara	FAR PO20
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Corona Angela	FAR PO09
Corona Paola	FAR PO02
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Costantino Gabriele	FAR PO10
	FAR PO17
	FAR OR14
Costantino Luca	FAR PO05
Costi Maria Paola	FAR OR16

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	FAR PO77
Ottonello Giuliana	FAR PO24
Paduano Francesco	FAR PO47
Pagano Francesco	FAR PO53
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Pagano Francesco*	FAR PO72
Pane Catello	FAR PO64
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Parodi Brunella	FAR PO42
Pasero Carolina*	FAR PO16
Peat Tom S.	FAR PO51
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Pedemonte Nicoletta	FAR PO24
Pedretti Alessandro	FAR OR24
Penna Ilaria	FAR PO24
Pepe Giacomo	FAR PO53
	FAR PO59
	FAR PO72
	FAR PO77
Peri Francesco*	FAR OR20
Perrin-Cocon Laure	FAR OR20
Perrone Mara	FAR PO35
Persico Marco	FAR PO33
Pescatore Luca	FAR PO41
Pesce Emanuela	FAR PO24
Petrelli Riccardo	FAR PO45
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Pfaff Tamara	FAR PO57
Piano Ilaria	FAR PO52
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Piccolo Marialuisa	FAR PO33
Picerno Patrizia	FAR PO64
Pieretti Stefano	FAR PO37
Piergentili Alessandro	FAR PO31
Pieroni Marco	FAR PO10
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Pieroni Marco*	FAR PO17
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Pinzi Luca	FAR PO82
Pippione Agnese Chiara	FAR OR12
Piragine Eugenia	FAR PO52
Pirali Tracey	FAR PO67
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Pirali Tracey*	FAR OR01
Piras Sandra	FAR PO02
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Pireddu Rosa	FAR PO65
Pirrello Giulia	FAR PO62
Pisani Leonardo*	FAR OR21
Pisapia Vincenzo	FAR PO07
	FAR PO21
Pittalà Valeria	FAR PO43
	FAR OR11
	FAR OR13
Pizzo Eliodoro	FAR PO03
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Polerà Nicoletta	FAR PO23
	FAR PO27
Polerà Nicoletta*	FAR PO18
Porco Melania Francesca	FAR PO69
Prezzavento Orazio	FAR PO43
	FAR OR11
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Prilc Sabrina	FAR PO08
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Prosdocimi Tommaso	FAR PO26
Protti Michele	FAR OR09
Pupo Giovanni	FAR PO09
	FAR PO80
	FAR OR15
Pupo Giovanni*	FAR PO41
Purgatorio Rosa	FAR PO61
Quaglia Fabiana	FAR PO03
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Quaglio Deborah	FAR PO38
Quattrini Luca*	FAR PO73
Quinn Mark T	FAR OR23
Raffa Demetrio	FAR PO29
Ragno Gaetano	FAR PO69
	FAR OR02
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Ramunno Anna	FAR PO33
Rapisarda Antonio	FAR PO68
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Rastelli Giulio*	FAR PO82
Rescigno Donatella	FAR PO14
Rescigno Donatella*	FAR PO19
Řezáčová Pavlina	FAR KN05
Riva Beatrice	FAR PO67
	FAR OR01
Rivara Silvia	FAR KN07
Rodriguez-Dorado Rosalia	FAR PO63
Rodríguez-Gimeno Alejandra	FAR PO24
Romeo Giuseppe	FAR PO43
	FAR OR11
Ropraz Patricia	FAR PO25
Russo Debora	FAR PO24
Russo Eleonora*	FAR PO42
Russo Mariateresa	FAR PO72
	FAR PO77
Russo Marina	FAR OR13
Russo Paola	FAR PO63
Russo Paola*	FAR OR19
Sabatini Stefano	FAR PO13
	FAR OR04
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Saccoliti Francesco	FAR PO09
	FAR PO41
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Saccoliti Francesco*	FAR OR15
Sainas Stefano	FAR OR12
Sala Marina	FAR PO47
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Sala Marina*	FAR PO74

Saladini Francesco	FAR OR05
Salerno Loredana*	FAR PO43
	FAR OR11
Salmaso Stefano	FAR KN06
Sanna Monica	FAR PO34
Sanna Monica*	FAR PO44
Sansone Francesca	FAR PO59
	FAR PO64
Santarem Nuno	FAR PO05
Santucci Matteo	FAR OR16
Sardella Roccaldo	FAR OR03
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Scala Maria Carmina	FAR PO47
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Scala Maria Carmina*	FAR PO75
Scandurra Roberto	FAR PO80
Schenone Silvia	FAR PO34
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Schepetkin Igor A	FAR OR23
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Siracusa Maria A	FAR PO43
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Sorrenti Valeria	FAR OR11
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Spadoni Gilberto*	FAR KN07
Spano Raffaele	FAR PO24
Spatari Claudia	FAR PO69
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Spensiero Antonia	FAR PO74
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Spyrakis Francesca*	FAR OR22
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Stefanucci Azzurra	FAR PO37
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Straniero Valentina*	FAR PO79
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Supuran Claudiu T.	FAR PO57
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Trist Iuni Margaret Laura	FAR PO44
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Tudino Valeria	FAR PO09
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Uliassi Elisa	FAR PO36
Ungaro Francesca	FAR PO03
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Valenti Donatella	FAR PO65
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Valoti Ermanno	FAR PO79
Vanella Luca	FAR OR11
Varra Michela	FAR PO33
Vázquez Eugènio	FAR OR10
Vellavita Rosa	FAR PO70
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Volpato Daniela	FAR PO30
Vullo Daniela	FAR PO55
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Wang Tung-Cheng	FAR OR20
Wolf Markus	FAR PO05
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Zaffaroni Lenny	FAR OR20
Zamperini Claudio	FAR PO16
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Zanusso Ilenia	FAR PO11
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