University *of Ljubljana*
Faculty *of Pharmacy*

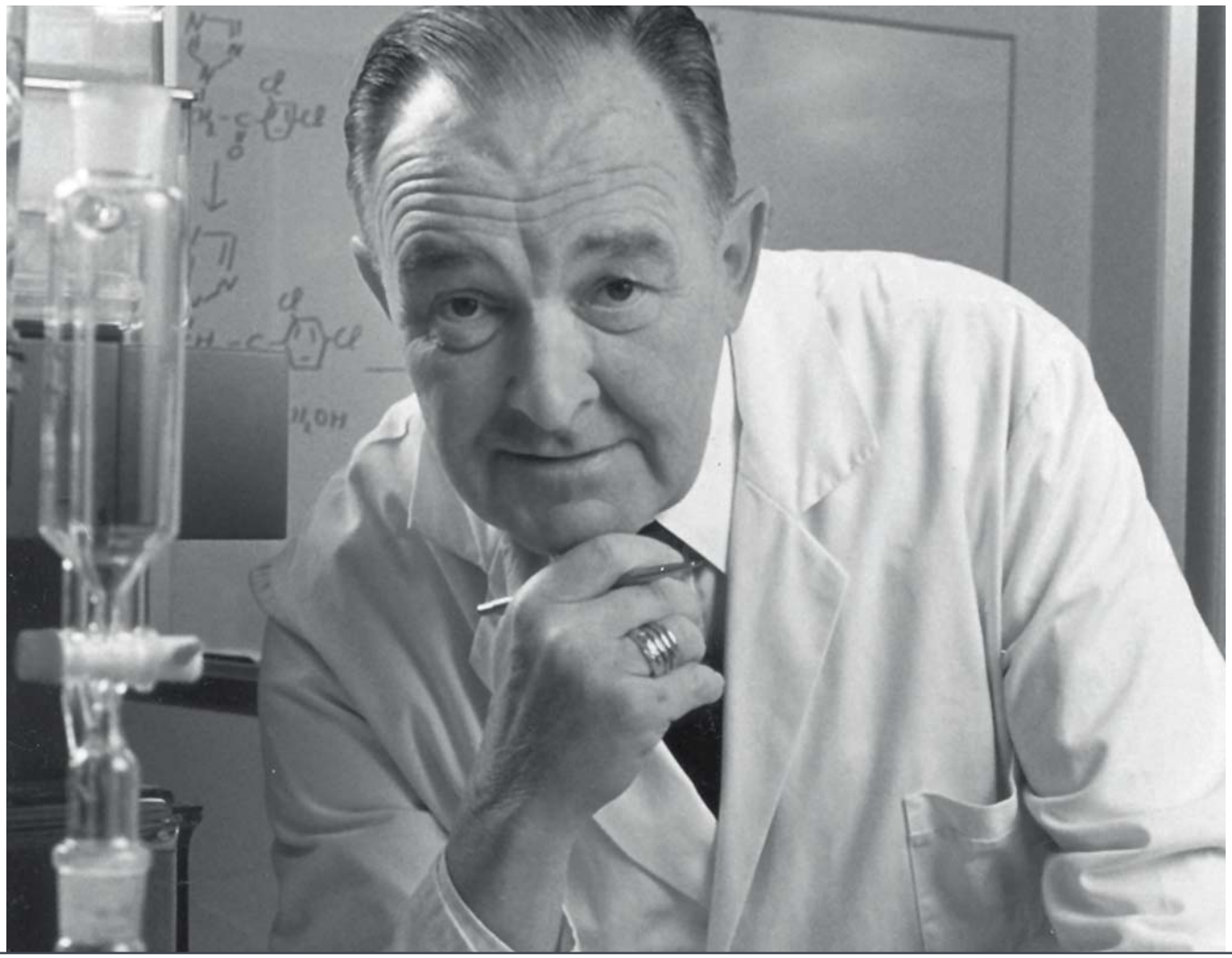
2nd Meeting of the Paul Ehrlich MedChem Euro-PhD Network

BOOK OF ABSTRACTS

9th-11th September 2012

Ljubljana, Slovenia

http://www.medchemeurophd2012.eu/



Dr. Paul Janssen

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University *of Ljubljana*
Faculty *of Pharmacy*

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Ljubljana, Slovenia

organized by

University of Ljubljana, Faculty of Pharmacy

Editors:

Assist. Dr. Tihomir Tomašić
Assoc. Prof. Lucija Peterlin Mašič
Assist. Prof. Janez Ilaš
Assist. Prof. Nace Zidar
Prof. Danijel Kikelj

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B. Kladnik
Ljubljana Tourism Archive

Dear Colleagues,

It is a pleasure for me to welcome you to the 2nd Meeting of the Paul Ehrlich MedChem Euro-PhD Network at the University of Ljubljana, Faculty of Pharmacy.

The Paul Ehrlich MedChem Euro-PhD Network was established in Palermo, Italy in November 2009 as a collaboration of 25 European universities with the aim of fostering the education and research training of post-graduate students in Medicinal Chemistry towards PhD degree. After a successful 1st Meeting of the Paul Ehrlich MedChem Euro-PhD Network hosted by three Madrid universities (Universidad de Alcala, Universidad Complutense de Madrid and Universidad San Pablo CEU) in July 2011, which brought together over 50 European PhD students in Medicinal Chemistry and about 40 senior researchers, belonging to 18 universities and research institutions from 6 European countries, the network has grown to 29 members and at this meeting we are hosting participants from 24 network member institutions and three non-network universities from 14 European countries, which gives the meeting a true international character.

The meeting will give PhD students in medicinal chemistry opportunity to present their research both in oral and poster presentations to an international auditorium of doctoral students and their supervisors from participating European countries and an excellent opportunity for scientific interactions with colleagues and senior scientists.

The Book of Abstracts of the 2nd Meeting of the Paul Ehrlich MedChem Euro-PhD Network contains the abstracts of 5 invited lectures presented by senior scientists from the network member institutions and pharmaceutical industry, as well as 40 oral presentations and 20 poster presentations contributed by PhD students from participating institutions. Over 100 meeting participants guarantee a critical mass for creative scientific interactions, new collaboration partnerships and wide dissemination of new ideas.

Wishing you a successful meeting and a pleasant stay in Ljubljana,

Danijel Kikelj

Meeting Chairman

International Scientific Committee (Network Executive Committee)

Prof. Giuseppe Ronsisvalle (Coordinator), University of Catania, Department of Pharmaceutical Sciences, Catania, Italy

Prof. Julio Alvarez-Builla, University of Alcalá, Department of Organic Chemistry, Alcala de Henares, Madrid, Spain

Prof. Norbert Haider, University of Vienna, Department of Drug and Natural Product Synthesis, Faculty of Life Sciences, Austria

Prof. Danijel Kikelj, University of Ljubljana, Faculty of Pharmacy, Slovenia

Prof. Péter Mátyus, Semmelweis University, Department of Organic Chemistry, Budapest, Hungary

Prof. Beatriz de Pascual-Teresa, University San Pablo CEU, Faculty of Pharmacy, Madrid, Spain

Organizing Committee

Prof. Danijel Kikelj (Meeting Chairman), University of Ljubljana, Faculty of Pharmacy, Slovenia

Assoc. Prof. Lucija Peterlin Mašič, University of Ljubljana, Faculty of Pharmacy, Slovenia

Assist. Prof. Janez Ilaš, University of Ljubljana, Faculty of Pharmacy, Slovenia

Assist. Prof. Nace Zidar, University of Ljubljana, Faculty of Pharmacy, Slovenia

Assist. Dr. Tihomir Tomašić, University of Ljubljana, Faculty of Pharmacy, Slovenia

GENERAL INFORMATION

Conference venue
University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia.
Lecture Hall P1.

Registration and information desk
Registration and information desk is located at the main entrance of the Faculty of Pharmacy.
Opening hours:

Sunday, September 9th	14:00 – 15:00
Monday, September 10th	8:30 – 9:00
Tuesday, September 11th	8:30 – 9:00

Badges
Badges will be given to every participant. All participants are required to wear the badges during the conference and social events.

Welcome reception
When: Sunday, September 9th at 19:00
Where: *Meta in bazilika* restaurant near the Faculty of Pharmacy

Ljubljana walking tour
When: Monday, September 10th at 19:00
Duration: 1 hour
Departure point: The Town Hall (Mestni trg 1)
The old city centre has a unique architectural appearance, particularly due to its mixture of Baroque and Art Nouveau architecture with masterful creations by the 20th century architect Jože Plečnik. The walking tour is organized for all registered participants. The tour starts in the Town Hall with a brief welcome address by the Mayor of Ljubljana and ends at the Ljubljana Castle in front of the restaurant *Gostilna na gradu*.

Paul Ehrlich Network dinner
When: Monday, September 10th at 20:00
Where: Ljubljana castle*,* restaurant *Gostilna na gradu*
You will enjoy meeting colleagues and friends in a special atmosphere of the Slovenian hospitality. In official part current status and plans for future development of the network will be presented and discussed. The traditional Slovenian food will be served.

Lunches
Lunches for registered participants will be served during the times indicated in this programme at the *Meta in bazilika* restaurant near the Faculty of Pharmacy. During the lunch time on Tuesday, September 10th, meeting of coordinators of the Paul Ehrlich Network will be organized.

Coffee breaks
Coffee and water will be served in front of the Lecture Hall P1.

Internet
Wireless internet access is available at the Faculty of Pharmacy for eduroam users. For others computers with internet access are available in the 1st floor of the faculty.

If you are a session chairperson, you are kindly asked to be in the Lecture Hall P1 at least 15 minutes prior to the beginning of the session to meet the speakers.
There are two types of lectures:

- invited lectures - 40 minutes, 5 minutes for discussion,
- oral communications – 13 minutes, 2 minutes for discussion.

The chairpersons are asked to strictly follow the scheduled times.

If you are a speaker, you are kindly asked to be in the Lecture Hall P1 at least 15 minutes prior to the beginning of the session to meet the chairpersons and the technician in charge of the projection. Authors can have their presentation on a CD-Rom or on an USB stick. You may supply your own laptop computer as a back-up. If combining video films with PowerPoint, please make sure to check it in the session hall during a coffee or lunch break prior to your session. Please, note that the conference computers in the session hall are being supplied with Windows XP and Office XP (at least).

Files must be uploaded on the central computer in the Lecture Hall P1 before the start of the session. It is recommended to upload your presentation before 9:00 for morning sessions and during lunch break for the afternoon sessions. A preview of the presentations will be possible during the breaks in the Lecture Hall P1.

If you are poster presenter, please see the list of posters in this program for the number assigned to your poster. The authors are kindly requested to put their posters on the display panels on Sunday during the registration and remove them at the end of the meeting. The mounting material will be available at the registration desk.

2nd Meeting of the Paul Ehrlich MedChem Euro-PhD Network Programme

9th September 2012

14:00 **Registration, Placement of Posters**

15:00 **Opening Ceremony**
Danijel Kikelj and Giuseppe Ronsisvalle

Session 1 – Chairperson: Danijel Kikelj

15:15 **IL-1 Opening Lecture**
Patrizio Mattei, F. Hoffmann-La Roche AG, Pharma Research & Early Development, Basel, Switzerland:
Discovery of carmegliptin: A potent and long-acting DPP-IV inhibitor for the treatment of type 2 diabetes

16:00 **Oral Communications**
Computational Approaches in Drug Design

O-1 *A. Cortes Cabrera, Universidad de Alcalá, Spain:*
OPTIMIZATION OF ACTIVE MOLECULES THROUGH EFFICIENCY PLANES USING THE ATLASCBS CONCEPT AND APPLICATION

O-2 *A. Introcaso, University of Bari, Italy:*
A MULTI-OBJECTIVE STRATEGY TO JOIN STRUCTURE- AND LIGAND-BASED DESIGN

O-3 *A. Arany, Semmelweis University, Hungary:*
MACHINE LEARNING BASED DATA FUSION FOR PRIORITIZATION OF DRUG CANDIDATES

O-4 *A. Artese, Università "Magna Græcia" di Catanzaro, Italy:*
COMPUTATIONAL STUDY FOR THE IDENTIFICATION AND STRUCTURAL CHARACTERIZATION OF NOVEL GENETIC ELEMENTS IN THE HIV-1 V3 LOOP REGULATING CO-RECEPTOR USAGE

17:00 **Coffee Break**

17:30 **Oral Communications**

O-5 *F. Morreale, Università di Messina, Italy:*
INSIGHT INTO THE FUNDAMENTAL INTERACTIONS BETWEEN LEDGF BINDING SITE INHIBITORS AND INTEGRASE COMBINING DOCKING AND MOLECULAR DYNAMICS SIMULATIONS

O-6 *P. Ježko, Comenius University Bratislava, Slovakia:*
THE PHARMACOPHORE MODEL DEVELOPMENT OF AT_1 ANGIOTENSIN II RECEPTOR ANTAGONISTS IN PROGRAM PHASE

O-7 *T. Vallianatou, University of Athens, Greece:*
QSAR ANALYSIS OF PPAR-α/γ ACTIVITY USING MULTIVARIATE STATISTICS AND MOLECULAR SIMULATION

O-8 *N. Manevski, University of Helsinki, Finland:*
GLUCURONIDATION OF INDOLES BY HUMAN UDP-GLUCURONOSYLTRANSFERASES 1A6, 1A9, and 1A10

O-9 *B. Brus, University of Ljubljana, Slovenia:*
NOVEL INHIBITORS OF TRIHYDROXYNAPHTHALENE REDUCTASE WITH ANTIFUNGAL ACTIVITY IDENTIFIED BY LIGAND-BASED AND STRUCTURE-BASED VIRTUAL SCREENING

19:00 **Welcome Reception**

10th September 2012

Session 2 – Chairperson: Péter Mátyus

09:00 **IL-2 Invited Lecture**
Christa Müller, University of Bonn, Pharmaceutical Institute, Bonn (Endenich), Germany:
Development of adenosine A_{2A} receptor antagonists - promising new therapeutics for neurodegenerative diseases

09:45 **Oral Communications**
Targeting GPCR

V. Rempel, PharmaCenter Bonn, Germany:
O-10 **3-BENZYLCOUMARINES: NOVEL LIGANDS FOR THE ORPHAN RECEPTOR GPR55**

K. Kamińska, Jagiellonian University Medical College, Poland:
O-11 **INFLUENCE OF SUBSTITUENT IN 6-POSITION OF 2-AMINO-4-(4-METHYLPIPERAZIN-1-YL)-1,3,5-TRIAZINE RING ON HISTAMINE H_4 RECEPTOR AFFINITY**

F. Mastronardi, Università degli Studi di Milano, Italy:
O-12 **SYNTHESIS OF NEW N-1 SUBSTITUTED 3-HYDROXY-PYRAZOLINE AMINO ACIDS SELECTIVELY INTERACTING WITH IONOTROPIC GLUTAMATE RECEPTORS**

U. M. Battisti, Università degli Studi di Modena e Reggio Emilia, Italy:
O-13 **5-ARYLBENZOTHIADIAZINE TYPE COMPOUNDS AS POSITIVE ALLOSTERIC MODULATORS OF AMPA/KAINATE RECEPTORS**

10:45 **Coffee Break and Poster Session**

Session 3 – Chairperson: Beatriz de Pascual-Teresa

11:15 **Oral Communications**
Synthetic Strategies

D. De Clercq, Ghent University, Belgium:
O-14 **SYNTHESES OF METHOTREXATE-HYBRID COMPOUNDS FOR TARGET PROFILING OF SMALL MOLECULES WITH MASPIT**

P. A. Ottersbach, University of Bonn, Germany:
O-15 **INDUCTION OF CHIRALITY: EXPERIMENTAL EVIDENCE OF ATROPISOMERISM IN AZAPEPTIDES**

O-16 *P. Bottino, University of Catania, Italy:*
STUDY OF TYPE 2 *TERT*-AMINO EFFECT

O-17 *J. Guillaume, Ghent University, Belgium:*
A DIVERGENT SYNTHESIS OF α-GALACTOSYLCERAMIDE ANALOGUES

O-18 *V. Estévez, Universidad Complutense de Madrid, Spain:*
SYNTHESIS OF A HIGHLY DIVERSE LIBRARY OF MEDICINALLY RELEVANT PYRROLES BY A NOVEL SYNTHETIC METHOD

O-19 *S. Álvarez Pérez, University San Pablo CEU, Madrid, Spain:*
SYNTHESIS OF NEW IMINOSUGARS WITH AN AZABICYCLE[4.1.0]HEPTANE STRUCTURE

12:45 **Lunch and Paul Ehrlich Network Meeting**

Session 4 – Chairperson: Julio Alvarez-Builla

14:15 **IL-3 Invited Lecture**
Elias Maccioni, University of Cagliari, Faculty of Pharmacy, Cagliari, Italy:
From classic to innovative approaches in HIV-1 infection treatment

15:00 **Oral Communications**
Anti-infectives

O-20 *M. Brvar, National Institute of Chemistry, Slovenia:*
STRUCTURE-BASED DESIGN OF 4,5'-BITHIAZOLE INHIBITORS OF DNA GYRASE B

O-21 *E. Arsovska, University of Ljubljana, Slovenia:*
DISCOVERY OF NEW ATP-COMPETITIVE INHIBITORS OF D-ALANINE:D-ALANINE LIGASE

O-22 *M. Hrast, University of Ljubljana, Slovenia:*
NEW CYANOTHIOPHENE INHIBITORS OF ESSENTIAL PEPTIDOGLYCAN BIOSYNTHESIS ENZYME MurF

O-23 *R. Oliveira, University of Lisbon, Portugal:*
ENDOPEROXIDE-BASED HYBRIDS CONTAINING FALCIPAIN INHIBITORS: SYNTHESIS AND ANTIMALARIAL EVALUATION

O-24 *J. Kratochvíl, Charles University, Czech Republic:*
SYNTHESIS OF 5-ALKYLIDENE-1,2,3,4-TETRAHYDROPYRIDIN-2-ONES RELATED TO GELASTATINS

16:15 **Coffee Break**

Session 5 – Chairperson: Norbert Haider

16:45 **Oral Communications**
Molecular Therapies for Inflammatory Diseases and Voltage-gated Sodium Channel Modulators

O-25 *P. Malerba, Università degli Studi di Bari, Italy:*
STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL DIARYL-PYRAZOLES AS COX-1 INHIBITORS

O-26 *L. Rycek, Vienna University of Technology, Austria:*
DEVELOPEMENT AND OPTMIZATION OF SYNTHETIC ROUTE TOWARDS UNSYMMETRIC MAGNOLOL DERIVATIVES, POTENTIAL CANDIDATES TO TREAT INFLAMATION

O-27 *Ž. Hodnik, University of Ljubljana, Slovenia:*
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF VOLTAGE-GATED SODIUM CHANNEL MODULATORS, BASED ON MARINE ALKALOIDS FROM *AGELAS* SPONGES

17:30 **Paul Ehrlich MedChem Euro-PhD Label Award**
Giuseppe Ronsisvalle

O-28 *B. Parrino, Università degli Studi di Palermo, Italy:*
MARINE ALKALOIDS ANALOGUES AS KINASE INHIBITORS

O-29 *M. R. Buemi, Università di Messina, Italy:*
RATIONAL DESIGN AND SYNTHESIS OF NEW GluN2B/NMDA RECEPTOR LIGANDS AS NEUROPROTECTIVE AGENTS

19:00 **Tour of Ljubljana**

20:00 **Paul Ehrlich Network Dinner at Ljubljana Castle**

11th September 2012

Session 6 – Chairperson: Giuseppe Ronsisvalle

09:00 **IL-4 Invited Lecture**
Beatriz de Pascual-Teresa, University San Pablo CEU, Madrid, Spain:
Molecular modeling in anti-cancer drug design and discovery

09:45 **Oral Communications**
Targeting DNA and Anti-cancer Drug Design

O-30 *R. Meleddu, University of Cagliari, Italy:*
9-FLUORENONES AS NEW SCAFFOLDS FOR G-QUADRUPLEX DNA

O-31 *F. Moraca, University "Magna Græcia" of Catanzaro, Italy:*
THE DISCOVERY OF NEW G-QUADRUPLEX BINDERS THROUGH A STRUCTURE-BASED VIRTUAL SCREENING APPROACH

O-32 *A. Brai, Università degli Studi di Siena, Italy:*
DISCOVERY OF MOLECULES INHIBITOR OF HUMAN DDX3 SPECIFICALLY DESIGNED TO TARGET THE RNA BINDING SITE

O-33 *S. Bhattarai, University of Bonn, Germany:*
BASE-MODIFIED ANALOGUES OF *α,β*-METHYLENE-ADP: STRUCTURE-ACTIVITY RELATIONSHIPS OF POTENT *ECTO*-5´-NUCLEOTIDASE INHIBITORS

O-34 *C. Ciancimino, Università degli Studi di Palermo, Italy:*
FUSED PYRROLO[2,3-*b*]PYRIDINE DERIVATIVES AS TOPOISOMERASE I INHIBITORS

O-35 *M. Gazvoda, University of Ljubljana, Slovenija:*
SYNTHESIS AND MODIFICATION OF BIOLOGICALY ACTIVE PYRAZOLONES

O-36 *A. L. Fallacara, Universitŕ di Roma, Italy:*
DEVELOPMENT AND OPTIMIZATION OF NEW COMPOUNDS AS ALLOSTERIC INHIBITORS OF Bcr-Abl KINASE

11:30 **Coffee Break and Poster Session**

Session 7 – Chairperson: Lucija Peterlin Mašič

12:00 **IL-5 Invited Lecture**

Stanislav Gobec, University of Ljubljana, Faculty of pharmacy, Ljubljana, Slovenia:

Discovery of new inhibitors of steroid metabolizing enzymes

12:45 **Oral Communications**

CNS Drug Research

O-37 *J. Korabecny, Charles University in Prague, Czech Republic:*
SYNTHESIS, BIOLOGICAL ASSESSMENT AND MOLECULAR MODELING OF NOVEL TACRINE-7-METHOXYTACRINE HETERODIMERS FOR ALZHEIMER DISEASE TREATMENT

O-38 *N. Guzior, Jagiellonian University Medical College, Poland:*
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DUAL BINDING SITE CHOLINESTERASE INHIBITORS WITH BETA AMYLOID ANTIAGGREGATION ACTIVITY

O-39 *M. Staderini, Universidad Complutense, Spain:*
STYRYLQUINOLINE DERIVATIVES POTENTIALLY USEFUL AS DIAGNOSTIC AND THERAPEUTIC AGENTS IN MISFOLDING DISEASES

O-40 *R. Turnaturi, University of Catania, Italy:*
DESIGN AND SYNTHESIS OF *trans*-DECAHYDROISOQUINOLINE DERIVATIVES AS NEW TRAMADOL-LIKE LIGANDS

13:45 **Closing Remarks**

14:00 **Lunch**

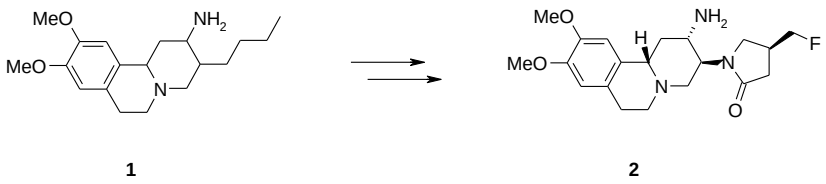
INVITED LECTURES

DISCOVERY OF CARMEGLIPTIN: A POTENT AND LONG-ACTING DIPEPTIDYL PEPTIDASE IV INHIBITOR FOR THE TREATMENT OF TYPE 2 DIABETES

P. Mattei[1,*]

[1]F. Hoffmann-La Roche AG, Pharma Research & Early Development (pRED), CH-4070 Basel, Switzerland

DPP–IV (dipeptidyl peptidase IV) inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP–IV rapidly cleaves and inactivates GLP–1, a stimulator of insulin secretion. Beneficial effects in diabetic patients through the preservation of active GLP–1 have been observed in clinical trials, and DPP–IV inhibitors appear to be devoid of the side effects associated with established classes of oral antidiabetic drugs, e. g., hypoglycemia, weight gain, edema, and gastrointestinal intolerance (1).



1 **2**

Fig. 1: From aminobenzo[a]quinolizine HTS hit 1 to carmegliptin.

The talk describes the design, synthesis, and SAR for a class of DPP–IV inhibitors that has emerged from **1** (Fig. 1), an aminobenzo[*a*]quinolizine derivative, which was found as a hit in a high-throughput screening campaign. Compound **1** had an interesting drug-like profile but needed improvement in terms of affinity with the target. The co-crystal structure of DPP–IV with **1** revealed a poor fit of the butyl side chain within the S1 specificity pocket of the enzyme and set the stage for the discovery of carmegliptin (**2**), a DPP–IV inhibitor which has recently completed phase 2 clinical trials (2).

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DEVELOPMENT OF ADENOSINE A_{2A} RECEPTOR ANTAGONISTS – PROMISING NEW THERAPEUTICS FOR NEURODEGENERATIVE DISEASES

C. E. Müller[1,*]

[1]Pharma-Zentrum Bonn, Pharmazeutisches Institut, Pharmazeutische Chemie I, An der Immenburg 4, D-53121 Bonn, Germany

Membrane receptors activated by purines are subdivided into three distinct families: (i) purine and/or pyrimidine nucleotide ("P2 receptors") receptors, further subdivided into G protein-coupled P2Y receptors ($P2Y_{1,2,4,6,11,12,13,14}$), and homo- or heterotrimeric ligand-gated ion channels or P2X receptors (subunits: P2X1-7); (ii) adenosine or P1 receptors (four subtypes: A_1, A_{2A}, A_{2B}, A_3), and (iii) P0 receptors activated by the nucleobase adenine (1). Purine receptors are widely distributed in the body and constitute novel drug targets. The xanthine alkaloids caffeine and theophylline, which are used as drugs for various indications, represent non-selective adenosine receptor antagonists (2,3) Epidemiological evidence has accumulated that coffee consumption is positively correlated with protection against neurodegenerative diseases, such as Parkinson's and Alzheimer's disease. Antagonists selective for adenosine A_{2A} receptors are in advanced clinical studies for the treatment of Parkinson's disease due to their positive effects on motoric symptoms in addition to disease-modifying neuroprotective properties (2).They may also be useful for the treatment of Alzheimer's disease, restless legs syndrome, depression, and addiction. In our group we have developed highly potent and selective A_{2A} antagonists, including water-soluble prodrugs and radiolabelled tool compounds, some of which are widely used in *in vitro* and *in vivo* studies (3).

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FROM CLASSIC TO INNOVATIVE APPROACHES IN HIV-1 INFECTION TREATMENT

E. Maccioni[1,*], S. Distinto[1], S. Alcaro[2], R. Meleddu[1], F. Esposito[1], A. Corona[1], M. L. Sanna[1], E. Tramontano[1]

[1]Department of Scienze della vita e dell'ambiente, University of Cagliari, via ospedale 72 09124 Cagliari, Italy; [2]Department of Scienze Farmacobiologiche, University of Catanzaro "Magna Graecia", 88021, Roccelletta di Borgia (CZ), Italy

Human immunodeficiency virus (HIV) is a retrovirus responsible of the acquired immunodeficiency syndrome (AIDS) (1,2). The syndrome consists of a progressive failure of the immune system leading to life-threatening opportunistic infections and cancers. According to UNAIDS (3) at the end of 2010, an estimated 34 million people [31.6 million–35.2 million] were living with HIV worldwide, up 17% from 2001. Moreover the number of people dying of AIDS-related causes fell to 1.8 million in 2010, down from a peak of 2.2 million in the mid-2000s. A total of 2.5 million deaths have been averted in low- and middle-income countries since 1995 due to antiretroviral therapy being introduced. Much of that success has come in the past two years when rapid scale-up of access to treatment occurred; in 2010 alone, 700 000 AIDS related deaths were averted. There are more than 20 antiretroviral drugs approved for the clinical treatment of HIV infected patients targeting different steps of the HIV replication cycle (4). The retrotranscription process is a key step in the early phases of HIV infection. It consists of the conversion of the viral ssRNA genome into a RNA-DNA hybrid and finally into an integration-competent dsDNA. This process requires both viral and cellular elements, among which the most important is the virus-coded RT protein. HIV-1 RT is a multifunctional enzyme with distinct associated activities, including RNA- and DNA-dependent DNA polymerase (RDDP and DDDP, respectively), ribonuclease H (RNase H), strand transfer, strand displacement synthesis and nucleotide excision (5-7). The two RT catalytic sites are inter-dependent and mutations in the polymerase domain affect the RNase H activity, and vice versa (8). The pivotal role of RT in the HIV-1 life cycle made it a druggable target for the AIDS chemotherapy. Therefore several classes of RT inhibitors (RTIs) have been approved for the treatment of HIV-1 infected patients (9,10). However, all RTIs in the market actually target only the RT-associated polymerase function (11). Hence, the identification of new RTIs, targeting other RT-associated functions, or more than one function, could represent an attractive challenge (12,13). Our research focus on the design of new dual inhibitors, capable to inhibit both polymerase and ribonuclease RT associated functions. The approach to the design of these molecules, their pharmacophoric requirements, and the devise of an efficient synthetic pathway to the desired structures as well as their activity and mechanism of action will be discussed.

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MOLECULAR MODELING IN ANTI-CANCER DRUG DESIGN AND DISCOVERY

B. Fabre[1], V. Roldós[1], P. Serra[1], J. M. Zapico[1], J. Hernández[1], S. Martín-Santamaría[1], A. Ramos[1], B. de Pascual-Teresa[1,*]

[1]Dpto. de Química, Facultad de Farmacia, Universidad San Pablo CEU, Madrid, Spain

Molecular modeling has been widely used for the understanding of relevant molecular recognition events. The appropriate and cyclic combination of different molecular modeling approaches together with synthetic procedures and followed by biological evaluation of the designed compounds has provided interesting hits and drug candidates targeting different macromolecules involved in cancer.

Some examples are reported that account for the application of molecular modeling to the study of matrix metalloproteinase inhibitors and PAMP modulators.

Among the matrix metalloproteinase family of enzymes, MMP1, 2 and 7 are the only ones that have been experimentally validated as cancer targets. MMP2 and MMP9 constitute the gellatinase subfamily and share many common structural features. However, MMP9 is considered as an anti-target enzyme in patients with advanced disease. For this reason, nowadays, in the design of MMP inhibitors (MMPi), a big challenge is to achieve MMP2 inhibition without affecting MMP9. In our research group we are currently working on different scaffolds for the design of MMP inhibitors.

PAMP (proadrenomedullin N-terminal 20 peptide) is a regulatory peptide that is detected in a large variety of cell types and exerts important biological activities. PAMP acts as a potent angiogenic factor and decorates microtubules in cells from different origins, controlling tubulin polymerization. A high-throughput docking-based virtual screening was performed, followed by a competitive monoclonal antibody assay on selected compounds, and a detailed ^{1}H, ^{15}N NMR spectroscopy study. This procedure has allowed us to describe the first small molecule capable of interacting with PAMP and potentially modulate its biological activity. Molecular modeling methods as docking and molecular dynamics were carried out to obtain a theoretical model of binding mode. Finally a directed in vivo angiogenesis assay (DIVAA) showed that the small molecule by itself has pro-angiogenic properties.

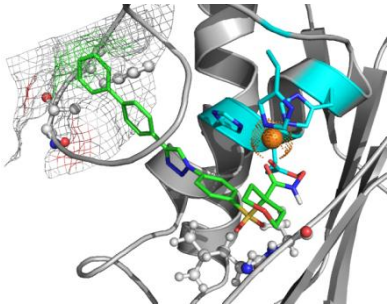

Figure 1. Minimized average structure from MD simulation of MMP2:inhibitor complex.

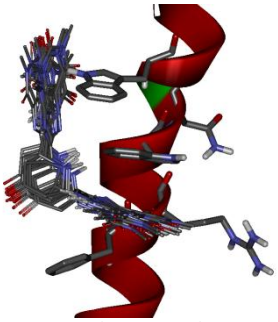

Figure 2. Docked conformations for **106221** bound to PAMP.

DISCOVERY OF NEW INHIBITORS OF STEROID METABOLIZING ENZYMES

S. Gobec[1,*]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

The discovery of new lead molecules with potential to interact with specific target receptors or enzymes is of central importance to early-stage drug discovery. The high cost and low hit rate of high-throughput screening have stimulated the development of computational (or *in silico*) screening methods (1). Virtual screening (VS) is an automated technique that is used for computational screening of large databases of compounds. Its aim is to reduce large numbers of compounds to smaller subsets that are more likely to contain biologically active compounds. Ligand-based VS uses 2D or 3D structure of active ligand and compares it with the structures of compounds present in a database. Structure-based VS applied to the discovery of new enzyme inhibitors involves docking, the computational fitting of structures of compounds to the active site of an enzyme, and scoring and ranking each compound. The highest ranked compounds are then tested for their activities in a biological or biochemical assay.

At the Faculty of Pharmacy, University of Ljubljana, we have used these *in silico* tools to search for small-molecule hit inhibitors of different pharmacologically important enzymes. Enzyme inhibitory activities were then evaluated in collaboration with the Institute of Biochemistry, Medical Faculty, where enzymes were also overexpressed, purified, and the enzyme inhibition assays were set up. Promising inhibitors of different steroid metabolizing enzymes like 17β hydroxysteroid dehydrogenase (2), aldo-keto reductases 1C1 and 1C3 (3), were discovered. These initial hit inhibitors represent an important starting point for development of new drug candidates for different diseases, including endometriosis, hormone dependent and hormone independent forms of cancer.

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ORAL COMMUNICATIONS

OPTIMIZATION OF ACTIVE MOLECULES THROUGH EFFICIENCY PLANES USING THE ATLASCBS CONCEPT AND APPLICATION

A. Cortes Cabrera[1,2,*], F. Gago[2], A. Morreale[1], C. Abad-Zapatero[1-3]

[1]Unidad de Bioinformática. Centro de Biología Molecular Severo Ochoa. 28049 Madrid, Spain
[2]Deptartment of Pharmacology. Universidad de Alcalá. Alcalá de Henares, 28871 Madrid, Spain
[3]Center for Pharmaceutical Biotechnology. University of Illinois at Chicago, Chicago, USA

Ligand efficiency (LE), generaly defined as the affinity of a ligand towards its biological target (given by Ki, IC50) scaled by its corresponding property (MW, No. of non-hydrogen atoms), is starting to gain acceptance in the medicinal and pharmaceutical chemistry communities as useful efficiency yardstick. Traditionally this is particularly true for ranking the efficiency of small molecules in approaches involving Fragment-Based libraries, but also for other, more general, medicinal chemistry projects.
A more encompassing definition of ligand efficiency was presented (1) that included two complementary Ligand Efficiency Indices (LEIs): BEI, SEI. The first one combines affinity with MW (BEI = pKi/(MW/1000) and the second one affinity with polarity (SEI = pKi/(PSA/100).
More generalized definitions of LEIs have been presented recently (2) suggesting that complementary pairs of variables (size, polarity) can be used to represent the drug chemico-biological spaces in an atlas-like representation (AtlasCBS).
We present here the AtlasCBS server application for molecular optimization in drug discovery using the framework of LEIs as variables. The efficiency of chemical compounds (in size and polarity) can be examined simultaneously in planes, guiding the optimization process to active molecules with the desirable physicochemical properties.
In addition, the AtlasCBS (3) allows to compare directly with a wide selection of compounds present in current chemico-biological databases such ChEMBLdb, PDBBind and BindingDB.
We propose that this representation of series of compounds and various Chemico-Biological Databases can be very useful to navigate the Chemico-Biological Space and could prove to be an invaluable tool to expedite drug discovery. The web site can be accessed at http://ub.cbm.uam.es/atlascbs.

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A MULTI-OBJECTIVE STRATEGY TO JOIN STRUCTURE- AND LIGAND-BASED DESIGN

A. Introcaso[1,*], O. Nicolotti[1], A. Carotti[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Bari, via Orabona 4, 70125 Bari, Italy

Drug discovery and development is a typical multi-objective task whose success depends on the simultaneous control of numerous, often conflicting, molecular and pharmacological properties (1). Earlier approaches in molecular optimization eluded this intrinsic complexity carrying out step-wise optimization of single properties. In this respect, multi-objective optimization (MOO) strategies represent a new approach to capture the occurrence of varying optimal solutions based on trade-offs among the objectives taken into account.
In the present work, the MOO was included into a genetic algorithm (GA), based on the principles of Darwinian evolution and survival of the fittest, with the purpose to improve interpretation of docking results looking for a balanced trade-off to the two conflicting terms of docking that are posing and scoring (2). The method was applied to the analysis of the well-studied series of 88 (72 in the training and 16 in the test set) benzamidine inhibitors with known activity for both thrombin and trypsin whose crystal structures are available from the Protein Data Bank.

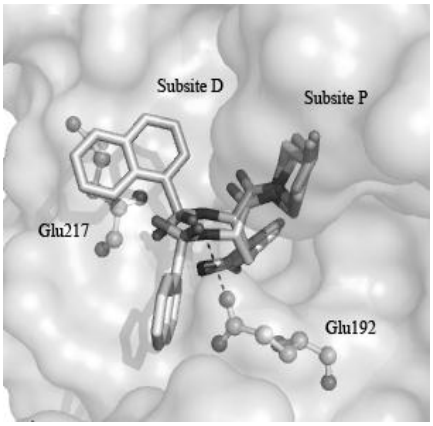

Fig. 1: Equivalent docking solutions in thrombin crystal structure (PDB code: 1ETS).

The MOO approach enabled the exploration of the trade-off between two key-point objectives in molecular design: the need of explaining the variance of biological affinity through linear regressions and the reproduction of the binding mode observed in the protein X-ray solved structures (3). Without requiring any a priori calibration and weighting scheme, MOO is simply based on a function of dominance to rank potential optimal models. Unlike single-objective optimization approaches, the pragmatic effect of MOO is, thus, the obtaining of a family of equivalent models representing different compromises for a given problem.

It goes without saying that MOO offered a number of explicit and valuable advantages compared to standard QSAR methods.
It was in fact demonstrated that MOO enabled the detection of the occurrence of equivalent, but different, binding conformations capturing diverse available potential sites of interaction within the protein binding site as shown in Fig. 1.
Finally, trade-off 3D QSAR models disclosed an outstanding sensitivity, improving docking screens, in recovering hits from a large chemical collection of decoys. The promise of a successful design of focused chemical libraries is the natural perspective of this study.

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MACHINE LEARNING BASED DATA FUSION FOR PRIORITIZATION OF DRUG CANDIDATES

A. Arany[1,*], B. Bolgár[2], P. Antal[2], B. Balogh[1], P. Matyus[1]

[1]Department of Organic Chemistry, Semmelweis University, Hőgyes Endre u. 7, 1092 Budapest, Hungary
[2]Department of Measurement and Information Systems, Budapest University of Technology and Economics, Magyar tudosok krt. 2, 1521 Budapest, Hungary

The available data about drug-like molecules are huge and more and more rich in modalities. Application of a data or knowledge fusion method is essential to organize and efficiently extract information. A special type of these databases can be used for repositioning of approved drugs to a new indication. Beside the classical chemical structure and target profile, phenotypic information sources (like side effects, approved indication, etc.) are also available in the above framework (1). Similar super-exponential growth of the available heterogeneous knowledge was observed in the field of molecular biology. Several methods have been developed in this context, which can be used in the field of medicinal chemistry to improve the efficiency of information fusion. In the field of genomics, a similar problem is candidate gene enrichment, where the main goal is to predict novel gene-disease associations *in silico* (2).

Our approach is based on an extension of SVM, a widely used machine learning algorithm with outstanding predictive performance. This method seeks to prioritize candidate drugs based on their similarity to a set of known active molecules (e.g. antiarrhythmics) using multiple information sources. We evaluated the method by predicting ATC (Anatomical Therapeutic Chemical Classification System) class memberships in a cross-validation scheme with high success rate.

Our future goal is to integrate this method in the classical medicinal chemical research pipeline to find potentially neuroprotective agents.

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COMPUTATIONAL STUDY FOR THE IDENTIFICATION AND STRUCTURAL CHARACTERIZATION OF NOVEL GENETIC ELEMENTS IN THE HIV-1 V3 LOOP REGULATING CO-RECEPTOR USAGE

S. Alcaro[1], A. Artese[1,*], G. Costa[1], F. Ortuso[1], L. Parrotta[1]

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

HIV-1 entry into host cells is a multistep process that requires coordinated interactions of the envelope glycoprotein gp120 with the CD4 receptor and with one of the chemokine receptors, CCR5 or CXCR4. Knowledge of how different genetic signatures of gp120 V3 domain affect the strength of these contacts is limited. The interaction between HIV-1 gp120 and CCR5 N terminus is critical for R5-virus entry and were evaluated by docking-analysis/molecular-dynamic simulations using AutoDock Vina (1) and Desmond (2) programs. Starting from the model described by Huang *et al.* (3), we generated all the mutants by single-residue replacement. The V3 genetic determinants significantly correlated with CCR5 or CXCR4 usage, and modulated gp120 affinity for CCR5 N terminus.

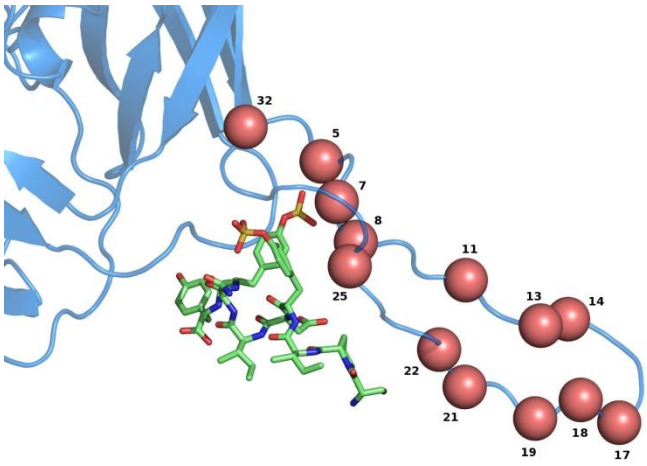

Fig. 1: Localization of V3 residues involved in coreceptor usage in the structure of HIV-1 gp120 domain.

Our simulations showed that other V3 genetic determinants, beyond the classical positions 11, 22 and 25, can modulate HIV-1 subtype B usage of CCR5 or CXCR4 coreceptor. Such an observation was detected by phenotype-genotype correlation and confirmed by structural analysis. This information can be used for finer tuning of potential efficacy of CCR5 and CXCR4 antagonists in clinical practice and to get molecular implications for the rational drug design of new entry inhibitors (4).

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INSIGHT INTO THE FUNDAMENTAL INTERACTIONS BETWEEN LEDGF BINDING SITE INHIBITORS AND INTEGRASE COMBINING DOCKING AND MOLECULAR DYNAMICS SIMULATIONS

F. Morreale[1,*], A. Chimirri[1], L. De Luca[1]

[1]Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, I-98168 Messina, Italy

Acquired immunodeficiency syndrome (AIDS) is the most challenging pandemic of the 21st century. Despite the increasing incidence of the disease, the prognosis of patients with AIDS has improved significantly over the last decade. Although the HAART has brought about a substantial decrease in the death rate and changed AIDS from a rapidly lethal disease into a chronic manageable condition, the retroviral infection can be only temporarily controlled but not eradicated. Furthermore, the HAART efficacy has been limited by the emergence of drug-resistant viral strains and drug-toxicity thus needing a change in treatment regimen. HIV-1 integrase (IN) has emerged as an attractive target for novel anti-AIDS agents. In particular nonactive-site-binding IN inhibitors would display synergy with current strand-transfer-specific IN inhibitors and other antiretroviral agents in clinical use. An effective allosteric inhibitory approach would be the disruption of interactions between IN and cellular cofactors. The association between IN and the cellular cofactor LEDGF/p75 is currently the most promising IN interaction for the design of protein–protein interaction (PPI) inhibitors in HIV-1 life-cycle (1).

In this study we investigated the fundamental interactions between five selected PPI inhibitors (2-4) and IN comparing them to the naturally occurring IN-LEDGF/p75 complex (5). Binding free energies were calculated using the molecular mechanics-generalized Born surface area (MM-GBSA) method, total energy was decomposed on per-residue contribution and hydrogen bond occupancies were monitored throughout the simulations. Considering all these results we obtained a good correlation with experimental activity and useful insights for the development of new inhibitors.

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THE PHARMACOPHORE MODEL DEVELOPMENT OF AT_1 ANGIOTENSIN II RECEPTOR ANTAGONISTS IN PROGRAM PHASE

P. Ježko[1,*], M. Remko[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 83232, Bratislava, Slovakia

System renin-angiotensin-aldosterone plays important role in regulation of blood pressure. The primary mediator of system renin-angiotensin-aldosterone is angiotensin II, which acts on its receptors AT_1 and AT_2. Antagonists of angiotensin AT_1 receptor (ARBs) are commonly used drugs for the treatment of hypertension, heart failure and renal disease. PHASE (1-3), a highly flexible system for common pharmacophore identification and assessment, has been used for pharmacophore model development of ARBs. A set of selective ARBs from BindingDB (4) has been selected for quantitative model generation. The obtained pharmacophore model has been validated. The best pharmacophore model of ARBs consists of six sites (ADHNRR): hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N) and two aromatic rings (R). Generated pharmacophore model of ARBs also contains distances and dihedral angles of pharmacophore sites. The results of this study can be used in the field of drug discovery, e.g. for virtual screening in large databases for potential drug candidates.

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QSAR ANALYSIS OF PPAR-α/γ ACTIVITY USING MULTIVARIATE STATISTICS AND MOLECULAR SIMULATION

T. Vallianatou[1,*], G. Lambrinidis[1], C. Giaginis[1,2], E. Mikros[1], A. Tsantili-Kakoulidou[1]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens 157 71, Greece
[2]Department of Food Science and Nutrition, University of the Aegean, Myrina, Lemnos 81400, Greece

Peroxisome proliferator-activated receptors alpha and gamma (PPAR-α, PPAR-γ) play a crucial role in the regulation of lipid and glucose metabolism. PPAR-γ has been extensively investigated as a target of a large number of anti-diabetic agents. However, as the simultaneous activation of PPAR-α is postulated to alleviate the side effects, related with PPAR-γ activation, research interest has recently been shifted towards dual PPAR-α/γ agonists. In the present study, Multivariate Data Analysis (MVDA) was combined with information on crystallographic data and molecular modeling, in order to investigate dual PPAR-α/γ activity (pEC_{50}-α and pEC_{50}-γ) using a data set of 71 carboxylic acid derivatives (1-5). Satisfactory PLS models were generated for each receptor subtype separately (R^2 =0.791, Q^2 =0.754 and R^2=0.782, Q^2=0.742 for PPAR-α and PPAR-γ activity, respectively). The models were based on simple and easily interpretable drug-like physicochemical and constitutional descriptors. By simultaneous analysis of both types of activity a consensus PLS model for dual PPAR-α/γ activity was established (R^2=0.755 and Q^2=0.713), displaying the molecular features, which may lead to a balanced activity. All models were validated by permutation tests and by external validation, dividing the data set into training and test sets. The differentiation of most important descriptors in the separate models, e.g. the higher impact of lipophilicity and bulk descriptors in PPAR-α and PPAR-γ activity respectively, as well as the effect of specific structural descriptors, were supported by detailed inspection of the relevant crystal structures and molecular simulation. The predictivity of the models was further evaluated by external blind test set (6). Good predictions were obtained for PPAR-α activity, while PPAR-γ activity was underestimated. This discrepancy was attributed to a specific structural characteristic, not included in the models, e.g, presence of two nitrogen atoms within ring (diazole ring). This feature seems to play a prevalent role in the case of PPAR-γ receptor, while not affecting binding on PPAR-α. The consensus PPAR-α/γ model resulted in better predictions in both cases, although underestimation of PPAR-γ activity persisted.

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GLUCURONIDATION OF INDOLES BY HUMAN UDP-GLUCURONOSYLTRANSFERASES 1A6, 1A9, and 1A10

N. Manevski[1,2,*], J. Troberg[2], J. Yli-Kauhaluoma[1], M. Finel[2]

[1]Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5, Helsinki, Finland
[2]Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5, Helsinki, Finland

Human UDP-glucuronosyltransferases (UGTs) are crucial phase II metabolic enzymes active in clearance of numerous endo- and xenobiotics from the body (1). Presently, despite considerable research efforts, glucuronidation of a novel compound cannot be predicted based on its chemical structure and thus requires time-consuming and expensive *in vitro* and *in vivo* experiments. The presented study tackles this problem and focuses on resolving one of the major issues within human drug metabolism: the complex and overlapping substrate specificity of UGTs. A set of indole-based molecular probes, comprised both of prominent drugs and experimental molecules is designed, synthesized and employed in the study of glucuronidation rates, enzyme kinetics, and binding affinities of these model substrates toward important drug-processing UGT isoforms 1A6, 1A9, and 1A10. Obtained data will be used for the refinement of a structural model of the UGTs substrate binding site and development of predictive QSAR models. The results could identify factors governing the substrate specificity and affinity for these important enzymes, facilitate structural studies of UGTs, and provide computational tools for a priori prediction of glucuronidation (*in silico* metabolic phenotyping). Acquired data could benefit the drug development process and enable design of specific substrates and inhibitors for individual UGT isoforms.

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NOVEL INHIBITORS OF TRIHYDROXYNAPHTHALENE REDUCTASE WITH ANTIFUNGAL ACTIVITY IDENTIFIED BY LIGAND-BASED AND STRUCTURE-BASED VIRTUAL SCREENING

M. Brunskole Švegelj[1], S. Turk[2], B. Brus[2,*], T. Lanišnik Rižner[1], J. Stojan[1], S. Gobec[2]

[1]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia
[2]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

Curvularia lunata is a dark pigmented fungus that is the causative agent of several diseases in plants and in both immunodeficient and immunocompetent patients (1). 1,8-Dihydroxynaphthalene-melanin is found in the cell wall of *C. lunata* and is believed to be the important virulence factor of dematiaceous fungi. Trihydroxynaphthalene reductase (3HNR) is an enzyme of the 1,8-dihydroxynaphthalene-melanin biosynthetic pathway, and it thus represents an emerging target for the development of novel fungicides and antimycotics (2). To date, only a few inhibitors of 3HNR are known. In the present study, we describe novel inhibitors of trihydroxynaphthalene reductase from *C. lunata*. These inhibitors were identified by ligand-based three-dimensional similarity searching and docking to a homology-built model and by subsequent biochemical and antifungal evaluation.

Among 47 tested compounds, five displayed significant inhibitory activity and the most potent one had IC_{50} value in high nanomolar concentration range. Compounds also affected the fungal growth and pigmentation. Although the antifungal activity test is not quantitative, a correlation between the IC_{50} values of compounds and their effects on growth and pigmentation of *C. lunata* was observed, which suggests that the antifungal activity seen is most probably a consequence of inhibition of 3HNR.

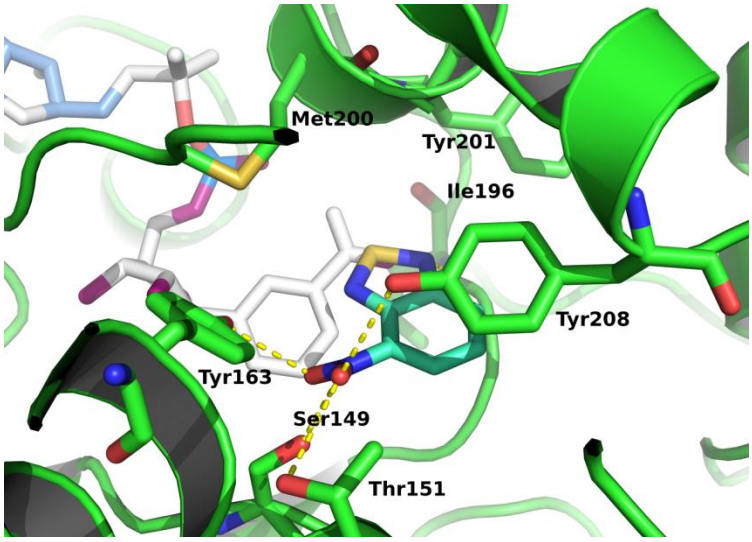

Fig. 1. Compound 44 (cyan) docked into the active site of 3HNR. The H-bonds with Ser149, Thr151, Tyr163, Ile196, and Tyr208 are shown as yellow dashes.

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3-BENZYLCOUMARINES: NOVEL LIGANDS FOR THE ORPHAN RECEPTOR GPR55

V. Rempel[1,*], N. Volz[2], F. Gläser[2], S. Bräse[2], C. E. Müller[1]

[1]PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany
[2]Institute of Organic Chemistry, University of Karlsruhe, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 6, D-76131 Karlsruhe, Germany

Cannabinoid (CB) receptors are involved in a variety of physiological and pathophysiological processes, for example the regulation of appetite and energy homeostasis, cognitive and mental functions, as well as mediation of pain and inflammation (1). To date, two cannabinoid receptor subtypes are known, CB_1 and CB_2, belonging to the GPCR superfamily of 7-transmembrane receptors (2). Recently, certain cannabinoid receptor ligands like the nonselective CB receptor agonist CP55,940 and the CB_1 selective inverse agonist rimonabant were found to interact with the orphan receptor GPR55, raising the question whether the GPR55 might be a third cannabinoid receptor subtype (3). We have recently described simple 3-benzyl-5-methoxycoumarin derivatives as new ligands for CB_1 and CB_2 receptors (4,5). In the present study we explored potential effects of coumarin derivatives at the related human GPR55 using β-arrestin translocation assays. Several derivatives were found to exhibit antagonistic properties. Our preliminry results indicate that 2-benzyl-5-hydroxycoumarines with 1,1-dimethylalkyl moieties in position 7 of the coumarin scaffold exhibit allosteric antagonism, while compounds lacking the alkyl residue in position 7 act as orthosteric antagonist (for structures see Fig. 1). The most potent and selective ligand identified so far displays an IC_{50} value of 1.88 µM and failed to interact with CB receptors. These initial structure-activity relationship studies should allow the design and development of more potent and selective GPR55 ligands in the future.

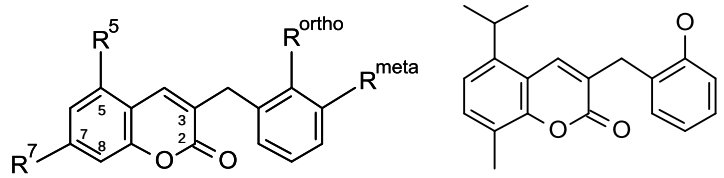

Fig. 1: Scaffold of the investigated coumarine deivatives at GPR55.

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INFLUENCE OF SUBSTITUENT IN 6-POSITION OF 2-AMINO-4-(4-METHYLPIPERAZIN-1-YL)-1,3,5-TRIAZINE RING ON HISTAMINE H_4 RECEPTOR AFFINITY

K. Kamińska[1,*], J. Ner[1], M. Więcek[1], T. Kottke[2], S. Schwed[2], R. Seifert[3], J. Handzlik[1], H. Stark[2], K. Kieć-Kononowicz[1]

[1]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland
[2]Institute of Pharmaceutical Chemistry, Johann Wolfgang Goethe University, Max-von-Laue Str. 9, 60438 Frankfurt, Germany
[3]Department of Pharmacology, Medical School of Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

There are four known receptors through which histamine mediates all its pharmacological functions in the body. These membrane-bound proteins - called histamine receptors and designated as H_1 to H_4 - belong to the family of G-protein coupled receptors. The object of our interest is the youngest member of the family – histamine H_4 receptor (H_4R), which was discovered and cloned in 2000/2001 by several independent research groups (1). It is suggested that H_4R is involved in inflammatory processes and immune responses, as its expression occurs mainly in monocytes, mast cells, dendritic cells, eosinophils and basophils (2). Therapeutic potential of H_4R antagonists can be drawn by the positive results on some animal diseases, e.g. allergic rhinitis, asthma or pruritus (3).

In our work, based on previous results and literature data (4,5) we are searching for new compounds with significant affinity to the H_4R in the group of 1,3,5-triazine derivatives. Presented research results involve newly obtained 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives modified in 6-position with different classes of substituents, which were divided into four groups: cycloaliphatic, substituted in 4-position aromatic, heteroaromatic and bicyclic substituents.

The compounds were obtained by the direct reaction of appropriate carboxylic esters with guanidine derivative. Subsequently they were evaluated for their affinity at human H_4R with radioligand binding assays using [^{3}H]histamine as radioligand. *In silico* predictions of toxicity and drug-likeness by newly obtained compounds by the OSIRIS program were also carried out.

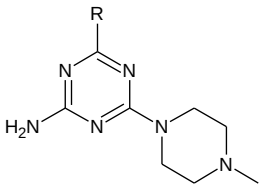

Fig. 1: General structure of the triazine derivatives studied.

The affinity of newly obtained compounds has shown great susceptibility to the kind of substituent in 6-position of the triazine ring and also to the pattern of this substituent modifications. The most potent compound showed K_i value of 137 nM.

This work was kindly supported by Polish Ministry of Science and Higher Education Grant No. 594-N/COST-2009/0, National Science Center DEC-2011/02/A/NZ4/00031, FP7 EU COST Actions BM0806 and BM1007.

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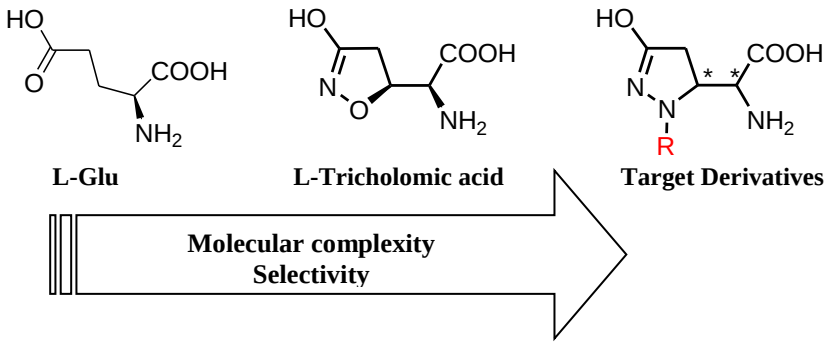
SYNTHESIS OF NEW N-1 SUBSTITUTED 3-HYDROXY-PYRAZOLINE AMINO ACIDS SELECTIVELY INTERACTING WITH IONOTROPIC GLUTAMATE RECEPTORS

F. Mastronardi[1,*], P. Conti[1], A. Pinto,[1] B. Nielsen,[2] D. Pickering,[2] C. De Micheli[1]

[1]Dipartimento di Scienze Farmaceutiche "P. Pratesi", Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[2]Department of Drug Design & Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

L-Tricholomic acid, a natural compound extracted from the poisonous mushroom *Tricholoma muscarium*, is a partially rigidified analogue of the endogenous ligand L-glutamate, where the distal carboxylic group is bioisosterically replaced by the 3-hydroxy-isoxazoline ring.
L-Tricholomic acid is an agonist at the AMPA and KA receptors while its D-enantiomer interacts selectively with the NMDA receptors; moreover both the L-threo and D-threo diastereoisomers are weak and non-selective GluR ligands (1).



Following the concept that increasing the molecular complexity may lead to increase the receptor selectivity, we replaced the 3-hydroxy-isoxazoline ring of the model compound L-Tricholomic acid with a 3-hydroxy-pyrazoline ring, which can be variously substituted at the N-1 position, inserting groups characterized by different electronic and steric properties.
Binding assays on slices of rat cerebral cortex showed that, depending on the nature of the substituent, some of the new synthesized ligands selectively interacted with either AMPA or KA receptors with affinities in the mid-micromolar range.
Selective KA receptor ligands were further characterized for their selectivity at homomerically expressed KA receptor subtypes.
Moreover, two derivatives, initially obtained as racemic mixtures, were resolved into the single enantiomers by preparative chiral HPLC and displayed a highly enantioselective interaction with KA receptors.

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5-ARYLBENZOTHIADIAZINE TYPE COMPOUNDS AS POSITIVE ALLOSTERIC MODULATORS OF AMPA/KAINATE RECEPTORS

U. M. Battisti[1,*], G. Cannazza[1], K. Jozwiak[2], G. Puia[3], G. Stocca[3], D. Braghiroli[1], C. Parenti[1], M. M. Carrozzo[4], C. Citti[4], L. Troisi[4], L. Brasili[1]

[1]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Modena e Reggio Emilia, Via Campi 183, 41125 Modena, Italy
[2]Laboratory of Medicinal Chemistry and Neuroengineering, Department of Chemistry, Medical University of Lublin, ul. W. Chodzki 4a, 20-093 Lublin, Poland
[3]Dipartimento di Scienze Biomediche, Università degli Studi di Modena e Reggio Emilia, Via Campi 287, 41125 Modena, Italy
[4]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, via Prov.le Lecce-Monteroni, 73100 Lecce, Italy

L-glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system (CNS) and its signal transduction is mediated by ionotropic and metabotropic receptors (1). Different studies suggest that ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAr) is involved in learning processes and in memory establishment (2). The therapeutic potential of compounds able to activate AMPAr has led to the search for new AMPAr positive modulators (3,4). Among these, one of the most investigated chemical class of compounds are benzothiadiazine derivatives such as cyclothiadiazide (CTZ), (±)7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (IDRA21) and (±) 8-chloro-2,3,5,6-tetrahydro-3,6-dimethyl-pyrrolo[1,2,3-de]-1,2,4-benzothiadiazine 1,1-dioxide that are able to inhibit desensitization of AMPAr potentiating ionotropic glutamatergic neurotransmission (Fig.1) (5-7). IDRA21, the first benzothiadiazine effective in increasing learning and memory performance in behavioral tests, represents an important lead compound since it is able to cross the blood-brain barrier (8). Basing on crystallographic data of the benzothiadiazines binding mode in the S1S2 GluA2 dimer interface, a set of 5-aryl-2,3-dihydrobenzothiadiazine type compounds, using IDRA21 as lead compounds, has been designed and synthesized. Hence, 5-aryl-7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide derivatives were prepared by a synthetic route based on the Pd-catalyzed Suzuki-Miyaura coupling reaction (Scheme 1). Electrophysiological results suggested that 5-heteroaryl substituents on benzothiadiazine core like 3-furanyl and 3-thiophenyl dramatically enhance the activity as positive modulators of AMPAr respect to IDRA21 and cyclothiazide (Table 1). Moreover mouse brain microdialysis studies have suggested that 7-chloro-5-(3-furyl)-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide crosses blood brain barrier after intraperitoneal injection. Biological results have been rationalized by a computational docking simulation that it has currently employed to design new AMPAr positive allosteric modulators.

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SYNTHESES OF METHOTREXATE-HYBRID COMPOUNDS FOR TARGET PROFILING OF SMALL MOLECULES WITH MASPIT

D. De Clercq[1,*], M. Risseeuw[1], S. Lievens[2], J. Tavernier[2], S. Van Calenbergh[1]

[1]Laboratory for Medicinal Chemistry, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[2]Cytokine Receptor Lab, Ghent University, Albert Baertsoenkaai 3, B-9000 Ghent, Belgium

Methods that allow high throughput identification of cellular targets of small molecules are valuable assets in pharmaceutical research. They are useful in mechanism of action studies of hits identified via phenotypic screening. Alternatively, they may uncover "off-target" proteins of established drugs that may contribute to their therapeutic efficacy. Finally, such methods also allow to profile small molecules against a series of related intracellular targets (e.g., kinases).

A recently developed assay, Mammalian Small molecule-Protein Interaction Trap (MASPIT) (1) provides a new tool for swift proteome-wide screening for intracellular targets of known small molecules. The principle of MASPIT is based on the JAK/STAT signaling pathway of the cytokine receptor (CR). A small molecule, or bait, is tethered to methotrexate (MTX) via a PEG linker. The MTX moiety allows immobilization of the bait onto the DHFR unit which is fused to the CR, thus allowing screening the bait against a collection of chimeric-prey proteins. Upon binding of the bait to a prey protein, the JAK/STAT signal cascade is activated, resulting in the expression of a luciferase reporter gene allowing a facile photometric read out.

We set out to synthesize alkyne-functionalized analogues of blockbuster drugs (simvastatin, propranolol and tamoxifen) and the small molecule reversine, paying close attention to SAR. These analogues were conjugated with an azide containing MTX-reagent via the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (CLICK chemistry). The resulting MTX-conjugates are currently being evaluated in the MASPIT assay.

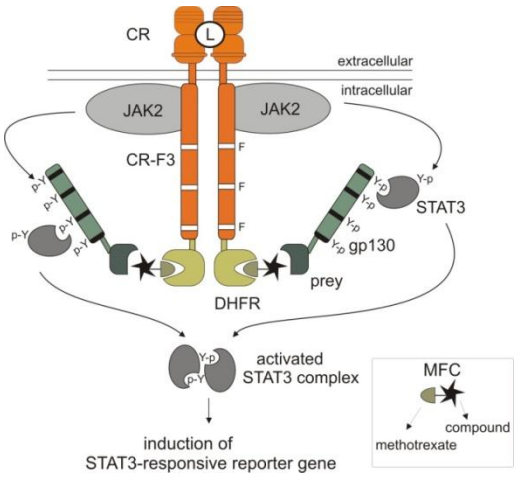

Fig. 1: Outline of the MASPIT system.

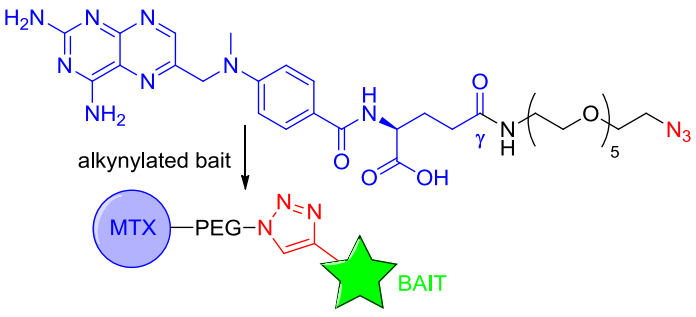

Fig. 2: General structure of MTX fusion compound prepared via CuAAC.

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INDUCTION OF CHIRALITY: EXPERIMENTAL EVIDENCE OF ATROPISOMERISM IN AZAPEPTIDES

P. A. Ottersbach[1,*], G. Schnakenburg[2] and M. Gütschow[1]

[1]Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany
[2]Institute of Inorganic Chemistry, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany

Azapeptides are peptides in which the $C_\alpha H$ unit of one or more amino acid(s) has been replaced by a nitrogen atom. This class of compounds has attracted much attention as bioactive peptidomimetics (1). The carbon-nitrogen replacement results in unique conformational preferences of the peptide. Hence, the analysis of the secondary structure of such molecules has gained relevance since the adoption of a specific conformation of (aza)peptides is crucial for biological recognition. Several attempts have been made to characterize conformational properties of azapeptides by theoretical techniques (2,3). However, considering their importance, the valuable crystallographic and spectroscopic investigations on azapeptides are rather limited (4).
In this work, structurally reduced azadipeptides with one amino acid (Cbz-Gly) connected to one aza-amino acid (aza-Gly-NH_2 or aza-Ala-NH_2) were examined by different NMR and crystallographic methods (5). Methylation of the connecting peptide bond leads to *E* configuration and hence to atropisomerism due to a restricted rotation around the N–N axis (Figure 1). The atropisomerism was diagnosed on the basis of the ^{1}H NMR signal, arising from diastereotopic Gly methylene protons, which was used as facile and suitable sensor of axial chirality. The occurrence of atropisomerism was verified by theoretical calculations of the energy barriers of rotation around the N–N axis and chiral chromatography.

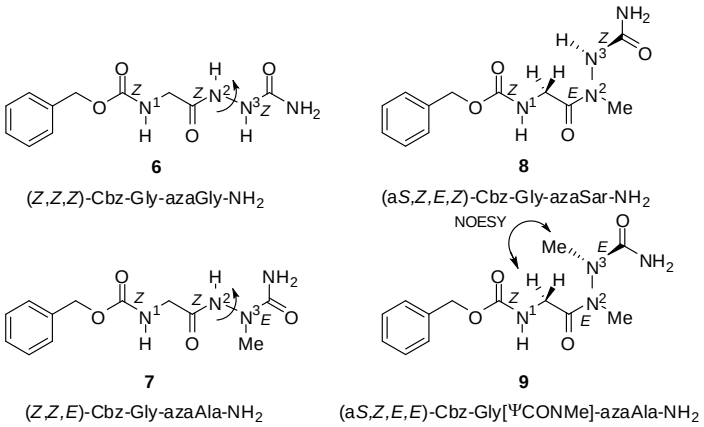

Fig. 1: Azadipeptide amides with free (6, 7) and restricted (8, 9; *S* enantiomers depicted) rotation around the N–N bond.

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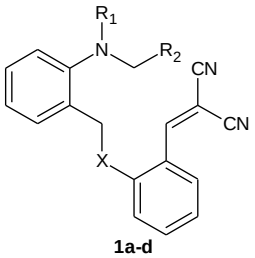
STUDY OF TYPE 2 *TERT*-AMINO EFFECT: NEW EXTENSIONS

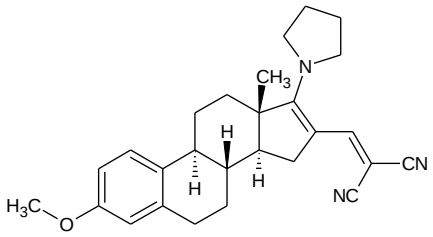
P. Bottino[1,2,*], G. Ronsisvalle[1], R. Ludmerczki[2], J. Wolfling[3], P. Matyus[2]

[1]Department of Pharmaceutical Sciences, University of Catania, Viale A.Doria 6, 95125, Italy
[2]Department of Organic Chemistry, Semmelweis University, H-1092 Budapest, Hõgyes E.u.7
[3]Department of Organic Chemistry, Szeged University, Dóm tér 8, H-6720 Szeged, Hungary

The term '*t* (later *tert*)-*amino effect*' was introduced by Meth-Cohn and Suschitzky (1) to generalize the thermal isomerization of *ortho*-substituted *tertiary* anilines to benzo-fused heterocyclic systems. In type 2 reactions, an *ortho*-vinyl *tert*-aniline gives a tetrahydroquinoline via C-C bond formation between the β-vinyl carbon and the α-methylene carbon of the amino group; the reaction could be extended to heterocyclic and bi- and tri-aryl analogues of anilines to obtain novel medium or larger ring systems (2,3). It has recently received much attention due to its wide scope and predictable regio- and stereoselectivity (4).
In the present work we report on our efforts to extend the type 2 *tert*-amino effect to non-conjugated systems, including: (I) biaryls connected with a saturated chain (compounds **1a-1d**, Fig. 1) and (II) steroids (represented, e.g. by **2**, Fig. 1).



1a-d



2

1a: R_1= CH_3, R_2 = H, X=NCH_3
1b: R_1= R_2= $(CH_2)_4$, X= NCH_3
1c: R_1= CH_3, R_2 = H, X=O
1d: R_1= R_2= $(CH_2)_4$, X= O

Fig. 1

In cases of **1a**, **1b**, the presence of two amino groups positioned *ortho* to a vinyl substituent in the same molecule, a priori, could lead to three different hydrogen migrations and, accordingly, different types of cyclizations. Although, isomerizations with the involvement of *N*-methyl group with a preference over the involvement of an *N*-benzyl might not be expected to take place at all, possible formations of six- and/or ten-membered rings (with N-benzyl carbons) could be considered. Not surprisingly, our isomerization experiment led to a six-membered ring. Next, as a fully unexplored field of *tert*-amino effect, steroids were investigated. We prepared compound **2**, properly functionalized in D-ring to study type 2 *tert*-amino effect in a non-aromatic system and utilize the isomerization reaction to open a new way to D-fused steroid-ring systems. Cyclization of **2** did indeed occur at high temperature to give two diastereomers in a ca. 1:1 ratio. Their separation is in progress.

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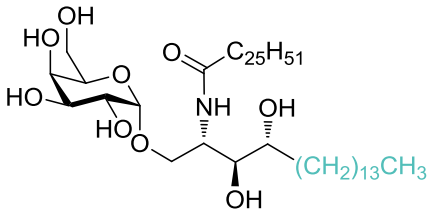
A DIVERGENT SYNTHESIS OF α-GALACTOSYLCERAMIDE ANALOGUES

J. Guillaume[1,*] and S. Van Calenbergh[1]

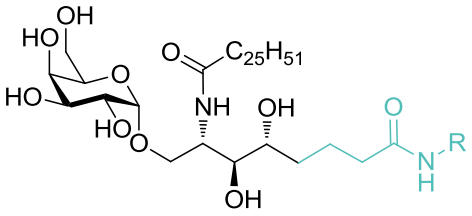
[1]Laboratory for Medicinal Chemistry (FFW), Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium

Screening of marine natural products for antitumor activities led to the discovery of glycolipids termed agelasphines, from which α-GalCer **1** was obtained by structural optimization. α-GalCer is presented by CD1d-molecules on antigen presenting cells to the TCR of *i*NKT cells, which upon activation rapidly secrete Th1 and Th2 cytokines. Th1 cytokines mediate protective immune functions like tumor rejection, antiviral and antibacterial effects, while Th2 cytokines mediate regulatory immune functions to ameliorate autoimmune diseases. The fact that Th1 and Th2 cytokines antagonize each other's effect is believed to be responsible for the limited clinical benefit obtained with α-GalCer (1). Hence, analogues that are capable of polarising the cytokine response to either Th1 or Th2 are of great interest.

In this poster a divergent synthetic pathway is described to afford α-GalCer analogues containing an amide moiety in the poorly investigated phytosphingosine chain. Recently, analogues with a pyrazole ring in the phytosphingosine chain showed a Th2 polarised cytokine response (2).



1 α-GalCer



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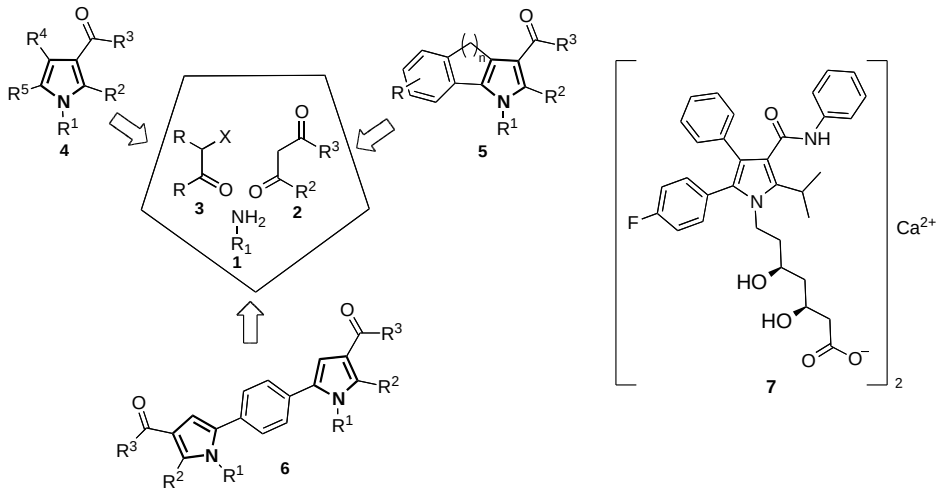
SYNTHESIS OF A HIGHLY DIVERSE LIBRARY OF MEDICINALLY RELEVANT PYRROLES BY A NOVEL SYNTHETIC METHOD

V. Estévez[1,*], M. Villacampa[1], J. C. Menéndez[1]

[1]Departamento Química Orgánica y Farmacéutica, Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Spain

Small molecules have a prioritary interest in the pharmaceutical market due their capability to influence the functionality of biological macromolecules. Diversity-Oriented Synthesis (DOS) is an approach proposed by Schreiber whose main objective is the development of divergent methodologies leading to highly diverse small molecules (1). Multicomponent reactions are an essential aspect of this approach. We consider the pyrrole nucleus as an appropriate scaffold to prepare structurally diverse libraries of potencially bioactive compounds, specifically antituberculosis candidates (2).

We report here our study of a Hantzsch-type reaction for the synthesis of pyrrole derivatives taking into account one of the general principles of green chemistry, the drastic restriction of the use of organic solvents. We used a new technique in organic synthesis based on mechanochemistry, known as high-speed vibrating milling (HSVM) (3). We carried out the reaction among primary amines **1,** β-dicarbonyl compounds **2** and α-haloketones **3** in the presence of a bicatalytic system formed by cerium(IV) ammonium nitrate (CAN) (4) and silver nitrate. Following this procedure, we prepared a large variety of polysubstituted pyrrole derivatives **4** as well as fused pyrrole **5** and bipyrrole **6** derivatives in order to test their activity against *Mycobacterium tuberculosis*. Finally, we report the application of the method to the synthesis of atorvastatin **7** (Lipitor®, Sortis®) (5), one of the top-selling drugs in the pharmaceutical market.



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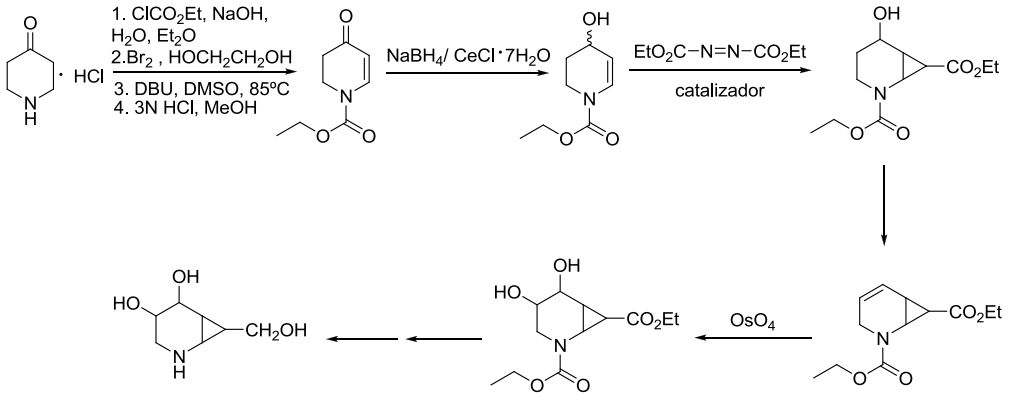
SYNTHESIS OF NEW IMINOSUGARS WITH AN AZABICYCLE[4.1.0]HEPTANE STRUCTURE

S. Álvarez Pérez[1,*], G. Domínguez Martín[1], J. Pérez Castells[1]

[1]Department of Chemistry, Faculty of Pharmacy, University San Pablo CEU, Urb. Montepríncipe, Ctra. Boadilla del Monte, Km. 5.300, 28668 Madrid, España

Iminosugars are natural or synthetic carbohydrate derivatives in which the endocyclic oxygen is substituted by a nitrogen atom. This substitution is related with the development of important changes into the biological properties of these kind of compounds (1). They act as glycomimetics and therefore, they are an interesting starting point for the synthesis of final products with relevant therapeutic interest. Consequently, new synthetic methodologies enroute to these aza-cycles are needed. Iminosugars generally comprise polyhydroxy 5- to 8-membered aza-rings. We have envisioned the utility of adding rigidity to the iminosugar structure by placing a cyclopropanic bridge in order to achieve selectivity against certain glycosidases. In this communication we present the synthesis of a new series of azabicycle[4.1.0]heptanes which are potentially active as glycosidase inhibitors, starting from an enaminone (2).



Scheme 1

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STRUCTURE-BASED DESIGN OF 4,5'-BITHIAZOLE INHIBITORS OF DNA GYRASE B

M. Brvar[1,*], A. Perdih[1], T. Solmajer[1]

[1]National Institute of Chemistry, Laboratory for Biocomputing and Bioinformatics, 1001 Ljubljana, Slovenia

DNA gyrase is a bacterial enzyme from the family of topoisomerases involved in the circular DNA molecule replication cycle. Its role is introduction of negative supercoils into the DNA molecule. It consists of two subunits A, with the DNA binding site, and two subunits B, possessing the ATP-ase activity (1). As it is present only in bacteria this enzyme represents a validated target for the design of novel antibacterials from the perspective of selective toxicity. Based on the available structural binding data of natural inhibitor clorobiocin (2) a pharmacophore model was constructed and using *in silico* approach a new class of DNA gyrase B inhibitors was discovered. Its antigyrase activity was measured using fluorescence-based enzyme assay and *in vitro* antibacterial activity on three different bacterial strains was determined. Based on the collected data promising hits were further validated utilizing several biophysical techniques including differential scanning fluorimetry (DSF), surface plasmon resonance (SPR) and microscale thermophoresis (MST). Additionally, x-ray structure of the complex between the G24 protein and the most potent inhibitor was determined showing good agreement with previously predicted binding mode and successfully confirming the ability of our model to identify novel DNA gyrase B inhibitors (3).

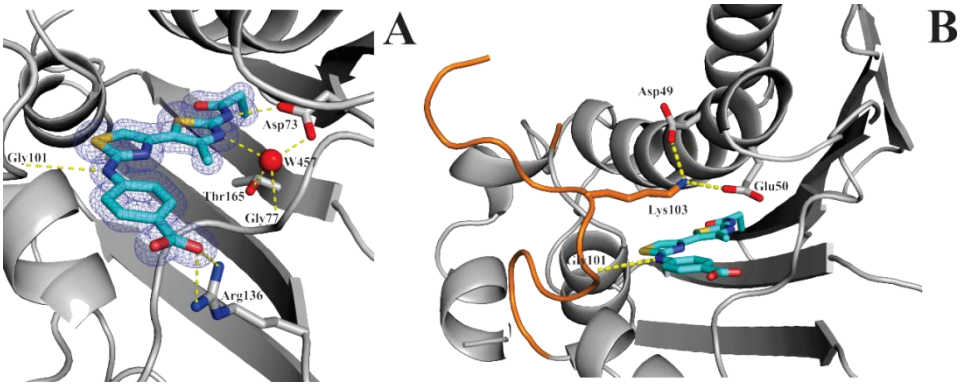

Fig. 1: (A) The position of the inhibitor in the active site of G24. Hydrogen bonds are presented with dotted lines; electronic density, contoured at 2 sigma, is shown as meshed net. (B) Interactions stabilising the flexible loop. Salt bridge formed between Lys103 and Asp49, Glu50 residues and hydrogen bond between the amine nitrogen of inhibitor and carbonyl group of Gly101 residue.

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DISCOVERY OF NEW ATP-COMPETITIVE INHIBITORS OF D-ALANINE:D-ALANINE LIGASE

E. Arsovska[1,*], V. Škedelj[1], T. Tomašić[1], A. Kroflič[2], V. Hodnik[3], M. Hrast[1], M. Bešter-Rogač[2], G. Anderluh[3], S. Gobec[1], J. Bostock[4], I. Chopra[4], A. J. O'Neill[4], C. Randall[4], A. Zega[1]

[1]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, Ljubljana, Slovenia
[2]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia
[3]Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia
[4]University of Leeds, Leeds, United Kingdom

The escalating evolution of bacterial resistance to the most of the currently available antibiotics leads to increased morbidity and mortality worldwide. So discovery of new antimicrobial agents with a novel mechanism of action and more chemical diversity has been one of the most important aims (1). Bacterial peptidoglycan is an essential cell wall polymer unique to prokaryotic cells that represents an interesting target for antibacterial drug design. D-alanyl-D-alanine ligase (Ddl) is one of the enzymes involved in peptidoglycan synthesis, catalyzes the ligation of D-Ala-D-Ala in the assembly of peptidoglycan precursors, and is considered as an important antimicrobial drug target (2). Ddl belongs to the ATP-grasp enzyme superfamily, which is characterized by an unusual nucleotide-binding fold, referred to as ATP-grasp fold. Sequence alignment of the enzymes from this enzyme superfamily revealed conserved motifs in the ATP-binding domain, suggesting that by targeting the ATP-binding site of bacterial members of the ATP-grasp superfamily, a multi-target antibacterial compound with the potential to reduce the development of the target based resistance can be designed (3). Encouraged by recent successful attempts to find selective ATP-competitive inhibitors of bacterial enzymes we designed, synthesized and evaluated a library of 6-arylpyrido[2,3-*d*]pyrimidine-based compounds as inhibitors of *Escherichia coli* DdlB (4). Inhibitor binding to the target enzyme was subsequently confirmed by surface plasmon resonance and studied with isothermal titration calorimetry. Since kinetic analysis indicated that 6-arylpyrido[2,3-*d*]pyrimidines compete with the enzyme substrate ATP, inhibitor binding to the ATP-binding site was additionally studied with docking. Some of these inhibitors were found to possess antibacterial activity against membrane-compromised and efflux pump-deficient strains of *E. Coli*. Best activity was achieved by compound with IC_{50} value of 133 μM.

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NEW CYANOTHIOPHENE INHIBITORS OF ESSENTIAL PEPTIDOGLYCAN BIOSYNTHESIS ENZYME MURF

M. Hrast[1,*], S. Turk[1], I. Sosič[1], D. Knez[1], C. P. Randall[2], H. Barreteau[3†], A. J. O'Neill[2], D. Mengin-Lecreulx[3], D. Blanot[3], S. Gobec[1]

[1]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
[2]Institute of Molecular and Cellular Biology and Antimicrobial Research Centre, University of Leeds, Leeds LS 9JT, U.K.
[3]Univ Paris-Sud, Enveloppes Bactériennes et Antibiotiques, IBBMC, UMR 8619 CNRS, 91405 Orsay, France
[†]Present address: Laboratoire Molécules de Communication et Adaptation des Microorganismes, UMR 7245 CNRS-MNHN, Muséum National d'Histoire Naturelle, CP54, 57 rue Cuvier, 75005 Paris, France

Peptidoglycan is an essential component of the bacterial cell wall, and its main function is the preservation of cell integrity by withstanding the internal osmotic pressure. The enzymes involved in peptidoglycan biosynthesis are among the best known and most extensively validated antibacterial drug targets. The D-Ala-D-Ala adding enzyme MurF catalyzes the final cytosolic peptidoglycan biosynthesis step: the addition of dipeptide to nucleotide precursor UDP-Mur*N*Ac-L-Ala-γ-D-Glu-*meso*-DAP (or L-Lys). As MurF has no human counterpart, it represents an attractive target for the development of new antibacterial drugs (1).
We designed, synthesized and biologically evaluated a new series of cyanothiophene-based inhibitors of MurF enzymes. The design was based on previously published Abbott inhibitors (2,3). Systematic structural modifications of these led to nanomolar inhibitors of *Streptococcus pneumoniae* MurF and micromolar inhibitors of MurF of *Escherichia coli* and *Staphylococcus aureus.* Some of the inhibitors also showed antibacterial activity against *S. pneumoniae R6* strain, and thus represent a good basis for further optimization towards an effective novel antibacterial drug.

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ENDOPEROXIDE-BASED HYBRIDS CONTAINING FALCIPAIN INHIBITORS: SYNTHESIS AND ANTIMALARIAL EVALUATION

R. Oliveira[1,*], J. Gut[2], P. J. Rosenthal[2], P. M. O'Neill[3], F. Lopes[1], R. Moreira[1]

[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Av.Prof. Gama Pinto, 1649-003 Lisbon, Portugal
[2]Department of Medicine, San Francisco General Hospital, University of California, San Francisco, Box 0811, San Francisco, California 94143
[3]Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, U.K.

The failure of classic antimalarial drugs such as chloroquine pushed research groups to find new therapeutic alternatives. Artemisinin (ART) and its semi-synthetic derivatives are now considered the last resource to fight multi-resistant malaria due to their outstanding activity (1). The drawback of being extracted from a limited natural source made necessary the discovery of synthetic analogues. Trioxolanes and tetraoxanes proved to be useful synthetic options to ART as a result of their similar activity and lack of toxicity (2,3). Falcipains are cysteine proteases essential for the survival of Plasmodium falciparum parasites. From the numerous falcipain inhibitors described in the literature (4), vinyl sulfones and aryl nitriles are among the most active scaffolds. Here we present the synthesis and antimalarial evaluation of two hybrid systems based on endoperoxides: the first coupled with vinyl sulfones and the second coupled with aryl nitriles (Fig. 1). The goal is to affect two targets within the parasite and to avoid the development of resistance to peroxide antimalarials. Preliminary data show that all tested hybrids display antiplasmodial activity in the low nanomolar range.

Fig. 1: General structure of the hybrids.

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SYNTHESIS OF 5-ALKYLIDENE-1,2,3,4-TETRAHYDROPYRIDIN-2-ONES RELATED TO GELASTATINS

J. Kratochvíl[1,*], E. Matoušová[1], O. Krenk[1], Z. Novák[1], J. Kuneš[1], M. Pour[1]

[1]Faculty of Pharmacy in Hradec Králové, Department of Inorganic and Organic Chemistry, Charles University in Prague, Heyrovského 1203, 500 02 Hradec Králové, Czech Republic

Compounds with 5,6-dihydro-2*H*-pyran-2-one skeleton occur in nature and possess wide range of biological activities from antibacterial effects to cytotoxicity (1). Given these interesting properties, our research in recent years was focused on gelastatins A and B (2). These molecules (isolated from *Westerdykella multispora*) have shown the ability to inhibit gelatinase A, a metalloproteinase participating on degenerative inflammatory processes and penetration of tumour cells into healthy tissues (3).
Over the past few years, our group has synthesized 3,5-disubstituted 5,6-dihydro-2*H*-pyran-2-ones with an alkylidene substituent at C5 (4), which possess interesting cytostatic activity. Regrettably, these compounds suffer from low stability. The purpose of this work has been to develop the synthesis of analogous 3,5- and 4,5-disubtituted lactams, which should be more stable and also more water-soluble than their lactone counterparts and investigate their chemical properties.

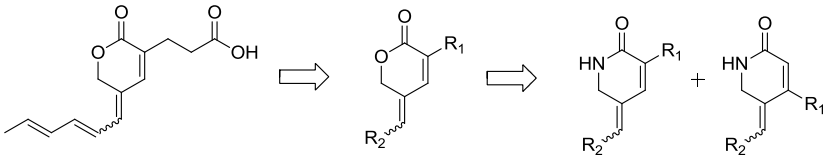

Fig. 1: Gelastatins and their potentially biologically active derivatives.

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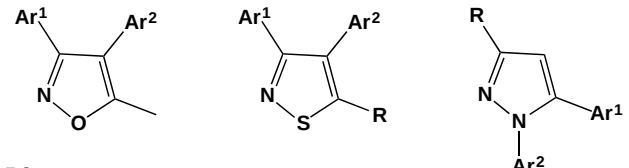
STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL DIARYL-PYRAZOLES AS COX-1 INHIBITORS

P. Malerba[1,*], M.G. Perrone[1], P. Vitale[1], A. Scilimati[1]

[1]Dipartimento di Farmacia, Università degli Studi di Bari "A. Moro", Via E. Orabona 4, 70125 Bari, Italy

Cyclooxygenase (COX) is the enzyme responsible for the conversion of arachidonic acid into prostanoids. Two cyclooxygenase isoforms are known, identified as COX-1 and COX-2.
COX-1 is considered a "housekeeping gene" due to the constitutive low-levels of expression in most cell types and tissues. High levels of constitutive expression of COX-1 have, instead, been detected in the stomach and platelets. In contrast, the gene for COX-2 is a primary response gene with many regulatory sites; thus, COX-2 expression can be rapidly induced by bacterial endotoxin LPS, cytokines, growth factors, and the tumor promoter phorbol myristate acetate.
In the last decade, abandoned the belief that the COX-1 inhibition was the unique cyclooxygenase isoform responsible of the side effects associated with (*t*-NSAIDs), COX-1 isoenzyme has been receiving increasing attention as a pharmacotherapeutic target, because of its involvement in many human diseases, such as atherosclerosis, endothelial dysfunction, neuro-inflammation, pain processing, pre-term labor, some type of cancers and gastrointestinal toxicity. Therefore, the development of selective COX-1 inhibitors might be highly relevant for the treatment of those pathologies. (1)
Among the diarylheterocycle COX inhibitor class, the isoxazole has been widely used as a central heterocycle ring for the preparation of potent and selective COX-1 inhibitors, such as **P6** (3-(5-chlorofuran-2-yl)-5-methyl-4-phenylisoxazole) (Figure 1). (2) The central role, if any, of the isoxazole nucleus in COX-1 inhibitors selectivity has been attempted to be clarified by preparing a set of new diarylheterocycles with different heterocyclic core rings (Fig. 1). The replacement of the isoxazole with an isothiazole (**1**) or a pyrazole (**2**) provided a drastic reduction in COX-1 inhibitory activity, while the introduction of an electron-donating group (EDG) on the *N*-aryl pyrazole allowed the recovery of COX-1 isoenzyme inhibitory activity and selectivity. (3)
Molecular docking studies of such novel pyrazoles into the binding site of COX-1 allowed also to shed light on its binding mode.
The synthetic methodologies, COX inhibitory potency and selectivity, and molecular docking findings will be presented.



P6 Ar^1 = 5-chlorofuryl
Ar^2 = phenyl

1

2

Fig. 1: Structures of P6 and novel diarylheterocycles with different heterocycles.

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DEVELOPEMENT AND OPTMIZATION OF SYNTHETIC ROUTE TOWARDS UNSYMMETRIC MAGNOLOL DERIVATIVES, POTENTIAL CANDIDATES TO TREAT INFLAMATION

L. Rycek[1,*], M. Draskovits[1], H. Stuppner[2], M. D. Mihovilovic[1]

[1]Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163-OC, A-1060, Austria
[2]Institute of Pharmacy/Pharmacognosy, University of Innsbruck, CCB, CCB - Centrum of Chemistry and Biomedicine, Innrain 80-82/IV, 6020 Innsbruck, Austria

Magnolol and structurally related honokiol are natural products from *Magnolia officinalis*, belonging to the class of neolignans. Recently, they have been shown to possess a variety of biological functions. Fakrudin et al. (1) showed that magnolol acts as a promising partial agonist of the PPARγ receptor and therefore it is a potential candidate for the treatment of diseases associated with the dysfunction of this nuclear transcription factor. PPARγ is above all a target for clinically used thiazolidinones (TZDs) to fight type II diabetes. Nevertheless, full activation, mediated by TZDs, can lead to many serious side effects, including weight gain, fluid retention or even heard failure. As was speculated recently, partial antagonist of PPARγ can eliminate some of them. Another physiological role of PPARγ is its involvement in inflammatory processes. Again, the development of partial antagonist is necessary in order to avoid the side effects of fully activated protein. In addition, magnolol was described as a strong $GABA_A$ agonist by Taferner et al. (2). Very recently also promising antibacterial and antiproliferative activities (3) of magnolol derivatives were described.
From the synthetic point of view, access to symmetrical magnolol molecule is relatively easy utilizing an oxidative coupling (4). However, access to unsymmetric derivatives remains challenging and versatile, and robust synthetic protocols are still lacking for such structures. We have therefore developed a versatile synthetic method based on transition metal catalyzed reactions. Our route enables easy access to a sub-type of unsymmetrical derivatives with one aromatic core containing the principal structural features of magnolol (allyl-substituent, phenolic OH-group) while simplifying the second aryl group with respect to the substitution pattern and functional groups. The optimization of the synthetic strategy will be elaborated within this contribution.

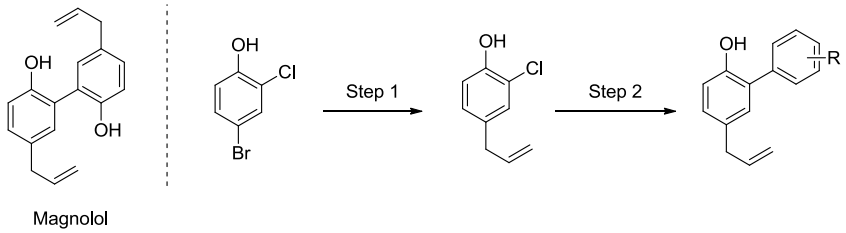

Fig. 1: Magnolol and the synthetic strategy for its asymmetrical derivatives.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF VOLTAGE-GATED SODIUM CHANNEL MODULATORS, BASED ON MARINE ALKALOIDS FROM *AGELAS* SPONGES

Ž. Hodnik[1,*], L. Peterlin Mašič[1], F. Chan[2], D. J. Madge[2], J. Tytgat[3] and D. Kikelj[1]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
[2]Xention Limited, Iconix Park, London Road, Pampisford, Cambridge CB22 3EG, UK
[3]Laboratory of Toxicology, University of Leuven, Onderwijs & Navorsing II, P.O. Box 922, Herestraat 49, 3000 Leuven, Belgium

Secondary metabolites produced by marine organisms usually exhibit great structural diversity, as well as high selectivity and specificity for binding to biological targets. These outstanding features make marine secondary metabolites promising source for discovery of new lead compounds (1). Besides many other marine organisms, sponges of the genus *Agelas* are a rich source of various alkaloids, e.g. clathrodin, hymenidin, dibromosceptrin, which show modulatory activity on voltage-gated sodium and calcium ion channels (2,3). Like most of the bioactive compounds of marine origin, alkaloids from sponges of the genus *Agelas* represent relatively complex chemical structures, whose total syntheses are complex, expensive and time consuming (4,5). Our aim was to design, synthesize and biologically evaluate compounds that will represent synthetically accessible analogs of clathrodin with improved activity on voltage-gated sodium channels. To examine the effect of (*E*)-5-(3-aminoprop-1-enyl)-1*H*-imidazol-2-amine moiety rigidification in clathrodin, we have designed, synthesized and biologically evaluated analogs of alkaloids of the genus *Agelas* that are based on 6-(aminomethyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine moiety, which also contains isosteric substitution of imidazolic nitrogen with sulfur atom (Fig. 1). 6-(Aminomethyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine moiety was then coupled with different carboxylic acids or its activated derivatives to investigate impact of pyrrole, bromopyrrole, dibromopyrrole, indole, proline and Boc-protected proline fragment in the molecule. Electrophysiological measurements of synthesized analogs on $Na_V1.1$–1.8 channel subtypes show that *tert*-butyl 2-(((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate exhibits 30% Ipeak block on $Na_V1.4$ channels in inactivated state at longer incubation times or increased Ipeak at shorter incubation times. Other synthesized compounds demonstrated weaker modulatory activity on all tested Na_V channel subtypes, while compound, with proline replacing pyrrole-2-carboxylic acid moiety, is still in the phase of biological evaluation.

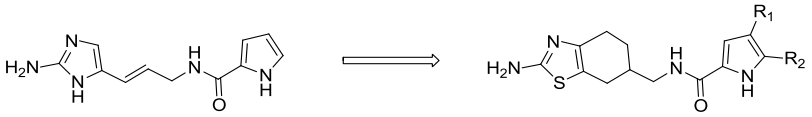

Fig. 1: The rigidification and modification of clathrodin.

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MARINE ALKALOIDS ANALOGUES AS KINASE INHIBITORS

B. Parrino[1]

[1]Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy

Indole alkaloids have received a great deal of attention because of their broad spectrum of biological activities including antimicrobial, antiviral and antitumor properties (1,2).
Nortopsentins A-C, having a characteristic 2,4-bis(3'-indolyl)imidazole skeleton, showed *in vitro* cytotoxicity against P388 cells (IC_{50} 1.7-7.8 μg/ml). Their N-methylated derivatives exhibit a significant improvement in P388 activity compared to that of the parent compounds (IC_{50} 0.34-0.90 μg/ml) (3). In our previous work we reported the synthesis of two new series of bis-indolyl-5-membered heterocycles in which the imidazole moiety of nortopsentin was replaced by thiophene and pyrazole rings. Some of these compounds showed antiproliferative activity with GI_{50} values in the micro- and sub-micromolar range (4,5).
Considering the interesting results obtained for previous series, we synthesized 3-[2-(1*H*-indol-3-yl)-1,3-thiazol-4-yl)-1*H*-4-azaindole derivatives, in which the 4-azaindole ring substituted one indole system and the thiazole moiety replaced the imidazole nucleus of nortopsentin, in order to verify whether the aza-substitution to the indole system increases the antineoplastic activity.
For all synthesized compounds *in vitro* screen based on the sulforodamine B (SRB) assay was performed in order to study the antiproliferative effects of all the synthesized compounds. Four compounds consistently reduced the growth of all experimental models independent of *TP53* gene status, with IC_{50} values ranging from 2.20±0.13 to 19.36±2.63 μM, and were also able to inhibit CDK1 activity in a cell-free assay with IC_{50}<1 μM. Further results will be discussed.

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RATIONAL DESIGN AND SYNTHESIS OF NEW GluN2B/NMDA RECEPTOR LIGANDS AS NEUROPROTECTIVE AGENTS

M. R. Buemi[1,*]

[1]Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, I-98168 Messina, Italy

Disorders in the central nervous system (CNS) are complex pathological states that represent one of the main public health problems for society. Multiple factors are crucial to the development of these disorders, including an imbalance in neurotransmitter receptor systems and a presence of free radicals. Glutamate (Glu) is the principal excitatory neurotransmitter in the CNS, where it is involved in physiological processes, but also in pathological states, including strokes, seizures, and pain. There are different classes of postsynaptic ionotropic glutamate receptors (iGluRs) (1), however selective antagonists targeting NMDA receptor subtype emerged as the most promising class of neuroprotective agents. They bind to so-called ifenprodil site exerting a blockade of receptor activation in a noncompetitive manner. In previous studies we reported a combined ligand- and target-based approach leading to the identification of a new class of potent ligands of NMDA receptor containing GluN2B subunit (2). The most active derivatives, containing indole scaffold (3), displayed high affinity, reduced NMDA receptor mediated current in patch clamp experiments and showed anticonvulsant efficacy (3). In attempt to obtain new ligands and further explore the key interactions within ifenprodil site of GluN2B-containing NMDA receptor, we synthesized a series of new compounds (Fig. 1) bearing various groups in the indole system, modified the linker between indole nucleus and piperidine/piperazine ring and introduced some modifications on benzylpiperidine fragment (4).

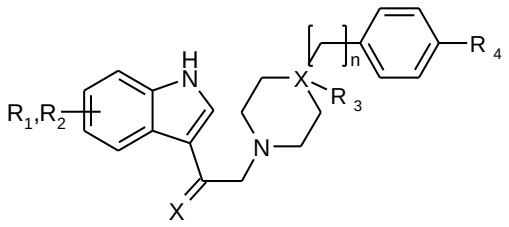

Fig. 1

For all synthesized compounds the affinity at the ifenprodil site has been evaluated by means of [^{3}H-ifenprodil] binding assays. Then we extended our studies carrying out electrophysiological experiments and examined *in vivo* efficacy against audiogenic seizures in DBA/2 mice. These studies were useful to highlight the main SAR for this class of ligands. Our findings were also corroborated by docking experiments which pointed out the main interactions within the binding site. Moreover, we planned the evaluation of the antioxidant ability of the new compounds containing free-radical scavenger moieties.

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9-FLUORENONES AS NEW SCAFFOLDS FOR G-QUADRUPLEX DNA

R. Meleddu[1,*], S. Distinto[1], L. Parrotta[2], M. L. Sanna[1], E. Maccioni[1], C. Musetti[3], C. Sissi[3], S. Alcaro[2]

[1]Department of Life and Environment Sciences, University of Cagliari, Via Ospedale 72, 09124 Cagliari
[2]Department of Healt Sciences, University of Catanzaro, Campus "Salvatore Venuta", Viale Europa, 88100 Catanzaro, Italy
[3]Department of Pharmaceutical Sciences, University of Padova, Via Marzolo 5, 35100 Padua, Italy

Since the identification G-quadruplex DNA assemblies of the G-rich sequence in the telomeric portion (1, 2), an increasing interest of the medicinal chemistry community has been devoted towards these structures as potential therapeutic targets. It is common knowledge that telomeres are specialized DNA–protein complexes at the end of eukaryotic chromosomes. They play an important role in the protection of chromosomal DNA from both hexogen and endogen derived damages. Noteworthy G-rich sequences are highly represented not only in telomeres but also in oncogenes promoter regions such as c-myc, c-kit, bcl-2, VEGF, H-ras and N-ras. Nowadays a large number of information is available on small molecules interacting with G-quadruplex. Both modelling and structural investigation have defined the main chemical requirements for effective G-quadruplex recognition by small molecules (3). So far G-quadruplex ligands exhibit a high potential as anti-tumour agents and some compounds have been considered for cancer therapy (Fig. 1).

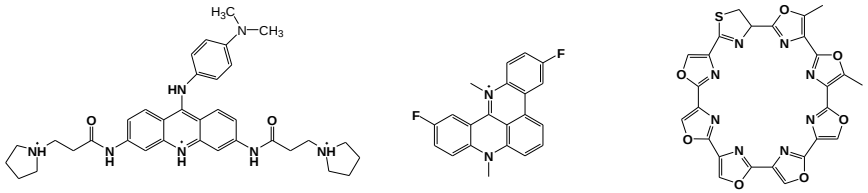

Fig. 1: Examples of G-quadruplex binders considered for cancer therapy.

Unfortunately none of these compounds reached the market, mainly due to the lack of drug-like properties.
In this poster we will present our studies on differently substituted 9-fluorenones. These molecules have been rationally designed starting from the results of a previous series (4) and their ability to stabilise G-rich sequences has been investigated. Some of these compounds exhibit an interesting biological activity and their potential as G-quadruplex binders will be discussed.

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THE DISCOVERY OF NEW G-QUADRUPLEX BINDERS THROUGH A STRUCTURE-BASED VIRTUAL SCREENING APPROACH

F. Moraca[1,*], S. Alcaro[1], A. Artese[1], G. Costa[1], S. Distinto[2], L. Parrotta[1], F. Ortuso[1]

[1]Department of Scienze della Salute, University "Magna Græcia" of Catanzaro, Campus "S. Venuta", Viale Europa, 88100, Catanzaro, Italy
[2]Department of Scienze della Vita e dell'Ambiente, University of Cagliari, Via Ospedale, 09124, Cagliari, Italy

DNA G-quadruplex (G4) structures are abundant in regions of biological significance, for example, at telomeres and in the promoters of many important genes (1). Stabilization of quadruplex architecture by small molecules is emerging as a potential anticancer approach since it is thought to interfere with oncogenic expression and telomeric maintenance in cancer cells (2). Its most active ligand, Telomestatin, has been extensively studied due to its outstanding selectivity for G-quadruplex (3). In recent years, the *structure-based design* approach has had a major impact on the rational design and optimization of new lead compounds in those cases where the receptor structure is well characterized (4). In this work, a virtual screening study, using the structure-based pharmacophore modelling approach, was applied on four G4/ligand complexes available in the Protein DataBank, in order to identify novel G4 binders. In addition, GBPM (Grid Based Pharmacophore Model) computational approach was carried out (5), and the ensemble docking studies were performed taking into account the Quadruplex polymorphism (3). The best scored compounds were selected for further biological studies.

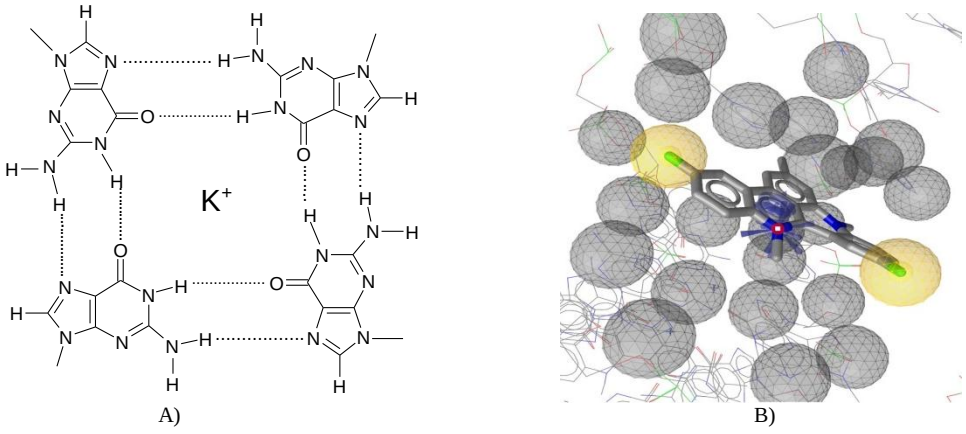

Fig. 1: A) The structure of G-Quadruplex guanine tetrads, with the K+ ion located at the center. B) A structure-based pharmacophore model built in this work.

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DISCOVERY OF MOLECULES INHIBITOR OF HUMAN DDX3 SPECIFICALLY DESIGNED TO TARGET THE RNA BINDING SITE

A. Brai [1,*], M. Selvaraj[1], R. Fazi[1], C. Tintori[1], J. Esté[2], A. Garbelli[3], G. Maga[3] and M. Botta[1]

[1]Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, via Aldo Moro, 53100 Siena, Italy
[2]Retrovirology Laboratory Irsi Caixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Barcelona, Spain
[3]Istituto di Genetica Molecolare, IGM-CNR, Via Abbiategrasso 207, 127100 Pavia, Italy

Compounds currently used for the treatment of HIV-1 infections are targeted to viral proteins. However, the high intrinsic mutation and replication rates of HIV-1 often lead to the emergence of drug resistant strains and consequent therapeutic failure. On this basis, the main argument in favor of targeting a host factor instead of a viral protein is the predicted low drug resistance level. One protein that has recently attracted much attention is DDX3. DEAD box polypeptide 3 (DDX3) is a DEAD box RNA helicase whose endogenous function is involved in mRNA splicing, export, transcriptional and translational regulation, and ribosome biogenesis. Interestingly, DDX3 appears to be a prime target for viral manipulation: at least four different viruses, namely Hepatitis C virus (HCV), Hepatitis B virus (HBV), Human Immunodeficiency Virus (HIV) and poxviruses, encode proteins that interact with DDX3 and modulate its function. HIV and HCV seem to co-opt DDX3 and require it for their replication. It has therefore been suggested that DDX3 could be a novel target for the development of drugs against these two viruses. DDX3 has multiple enzymatic activities (ATPase and RNA helicase) and functional domains that may be targeted by potential inhibitors.
Some compounds identified through computational studies have been synthesized and sent to the biological assays. Two of the selected compounds showed submicromolar activity towards the helicase binding site of DDX3.

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BASE-MODIFIED ANALOGUES OF *α,β*-METHYLENE-ADP: STRUCTURE-ACTIVITY RELATIONSHIPS OF POTENT *ECTO*-5′-NUCLEOTIDASE INHIBITORS

S. Bhattarai[1,*], A. El-Tayeb[1], M. Freundlieb[1], C. E. Müller[1]

[1]PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

The *ecto*-5′-nucleotidase (*ecto*-5′-NT, *e*N) is a member of the family of *ecto*-nucleotidases which dephosphorylate extracellular nucleotides, the others being the nucleoside triphosphate diphosphohydrolases (NTPDases; subtypes 1, 2, 3 and 8), the nucleotide pyrophosphatases (NPPs 1-3) and the alkaline phosphatases (APs; tissue non-specific, intestinal, placental and germ cell). Inhibitors of NTPDases and NPPs lead to an increase in the extracellular concentrations of nucleotides and thereby potentiate purinergic signaling via P2 receptors (1). *Ecto*-5′-NT inhibitors reduce extracellular adenosine levels, resulting in an indirect blockade of adenosine (P1) receptor activation. *Ecto*-5′-NT inhibitors have therefore potential as novel drugs, e.g. for cancer therapy (2). In contrast to direct receptor ligands, the enzyme inhibitors are indirect antagonists, which will exhibit site- and event-specific effects. *α,β*-methylene-ADP (AOPCP) is one of the most potent inhibitors of *ecto*-5′-NT; it exhibits a competitive mechanism of inhibition (3). In the present study we designed and synthesized various base-modified analogues of AOPCP with the goals (i) to study their structure-activity relationships at *ecto*-5′-NT, (ii) to obtain more potent and selective *ecto*-5′-NT inhibitors, and (iii) to design and synthesize metabolically stable AOPCP analogues, which cannot be metabolized *in vivo* to adenosine/adenosine receptor ligands. An initial set of 10 analogues of the potent *ecto*-5′-NT inhibitor AOPCP was synthesized. These compounds were obtained by a convergent synthesis in 7 steps with overall yields of 40-75%. The developed synthetic strategy is straightforward and allows for broad structural modifications. All products have been tested at rat *ecto*-5′-NT and found to exhibit higher inhibitory potency than that of AOPCP; e.g., the N^6-di(ethyl)substituted derivative and the N^6-di(methyl)substituted derivative showed K_i values of 0.060 μM and 0.080 μM, respectively, as compared to a K_i value of 0.190 μM for AOPCP tested under the same conditions.

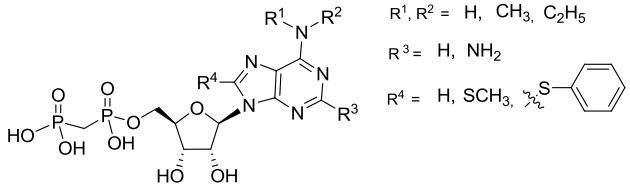

Fig. 1: Structures of synthesized AOPCP analogues.

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FUSED PYRROLO[2,3-*b*]PYRIDINE DERIVATIVES AS TOPOISOMERASE I INHIBITORS

C. Ciancimino[1,*]

[1]Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy

Cancer always constitutes one of the principal causes of death; so it is necessary to find out new drugs to introduce in therapy. Polycyclic nitrogen heterocycles can be good pharmacophores for classes of antineoplastic drugs because of their potential ability to bind to DNA by intercalating between the base pairs of the DNA duplex. Many 1,2,3-triazine and cinnoline derivatives are well known compounds endowed with a wide range of biological properties such as antineoplastic activity (1-4).
In our attempts of looking for novel antitumor agents, we extended our interest to the 7-azaindole[1,2-c][1,2,3]benzotriazines and 7-azaindole[3,2-*c*]cinnolines with the aim of evaluating their antitumor activity.
Five derivatives tested by the National Cancer Institute exhibited antitumor activity against the total number of the 60 cell lines panel from micromolar to nanomolar concentrations. Flow cytometry experiments were also performed in order to evaluate the mode of cellular death.
Results showed that these compounds are able to induce cell death by apoptosis involving some cellular organelles, such as mitochondria.
Further studies were also performed in order to clarify their mechanism of action. In particular, DNA cleavage reactions with human topoisomerase I were carried out in presence of various concentrations of compounds to verify a possible inhibition of the enzyme activity. Results suggested that these molecules act as poisons of topoisomerase I.

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SYNTHESIS AND MODIFICATION OF BIOLOGICALY ACTIVE PYRAZOLONES

M. Gazvoda[1,*], B. Burja[1], M. Kočevar[1], S. Polanc[1]

[1]Faculty of Chemistry and Chemical Technology, Department of Organic Chemistry, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia

Microtubules, dynamic filamentous cytoskeletal proteins composed of tubulin, have several key roles in cell, including in cell proliferation where they play important role in the process of mitosis (1). Their importance in mitosis and cell division makes microtubules an important target for anticancer drugs. It was previously belived that microtubule-targeted drugs work primarily by increasing or decreasing the cellular microtubule mass, although these effects might have a role in their chemotherapeutic actions, we now know that at lower concentrations, microtubule-targeted drugs can suppress microtubule dynamics without changing microtubule mass – this action leads to mitotic block and apoptosis (2). Some microtubule-targeted drugs can act as vascular-targeting agents which rapidly depolymerize microtubules of newly formed vasculature and shut down the blood supply to tumours – among them is also combretastatin A-4 (CA-4), natural stilbenoid phenol (3). Our recently disclosed simple and general route to pyrazol-3-ones via cinnamic acids, hydrazides and diazenes offers interesting gateway for the synthesis of different types of biologically active compounds (4). In recent publication we described synthesis and biological activity of pyrazolone-fused combretastatins and their precursors with some excellent anti-cancer activity (5). The pyrazolone-fused combretastatin A-4 analogues and hydrazides (see Figure below) are highly cytotoxic against various tumor cell lines, including cisplatin resistant cells. Described compounds also turned out to be good inhibitors of tubulin polymerization. Details and some recent efforts to increase cytotoxicity will be presented.

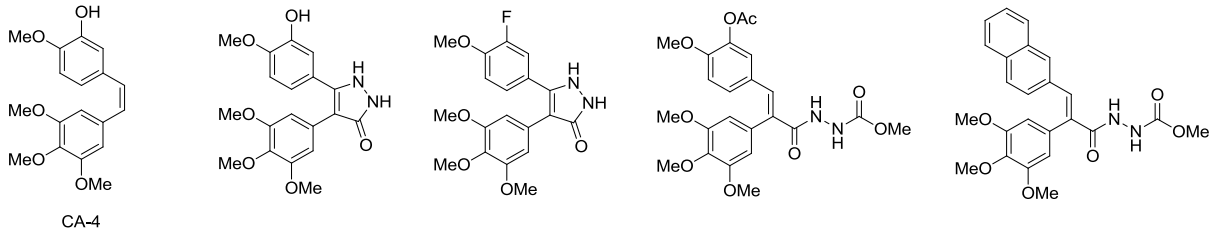

Fig. 1: Structure of combretastatin A-4 (CA-4) along with its pyrazolone-fused and hydrazide analogues.

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DEVELOPMENT AND OPTIMIZATION OF NEW COMPOUNDS AS ALLOSTERIC INHIBITORS OF BCR-ABL KINASE

A. L. Fallacara[1,*], C. Tintori[1], M. Radi[1], S. Schenone[2], E. Crespan[3], G. Maga[3], M. Botta[1]

[1]Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Via Aldo Moro, Siena, Italy
[2]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Genova, Viale Benedetto XV,3, Genova, Italy
[3]Istituto di Genetica Molecolare IGM-CNR, Via Abbiategrasso 207, 27100 Pavia, Italy

Chronic myelogenous leukemia (CML) is an hematological disorder caused by a fusion protein, Bcr-Abl, with deregulated tyrosine kinase activity. Imatinib mesylate (Gleevec; Novartis, Basel, Switzerland), an ATP-competitive Abl kinase inhibitor, is currently used as frontline therapy. However, patients treated with Imatinib (IM) can develop resistence because of the onset of amino acid point mutations within the kinase domain. Although second generation Bcr-Abl inhibitors are able to inhibit most of the IM-resistant forms of Bcr-Abl, neither compound is capable of inhibiting the "gatekeeper" T315I mutation. Recently, the discovery of GNF-2(1), a selective allosteric Bcr–Abl inhibitor, was reported. GNF-2 was found to bind to the myristate pocket located at the C-terminus domain of the Abl kinase. In the search for novel scaffolds to be tested as Bcr-Abl inhibitors, some interesting thiadiazole derivatives with submicromolar inhibitory activity against Abl were identified (2). Among these compounds BO-1 attracted our attention. In fact, it showed an interesting inhibitory activity against both Abl wt and T315I mutated form (K_i=0.10 μM and K_i=0.40 μM, respectively). Furthermore, a kinetic enzymatic study showed that BO-1 is an ATP-competitive inhibitor of Abl-wt while it acts as a non-competitive inhibitor in the case of Abl T315I. Docking simulations and Molecular Dynamics were performed by screening an internal library of pyrazolo[3,4-d]pyrimidines derivatives on both ATP and myristate binding sites in order to identify the main features of the structure involved in the binding within the two pockets. The aim of our work is to develop compounds characterized by an improved activity profile toward the T315I mutant. In this regards, some interesting scaffolds have been already identified by us.

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SYNTHESIS, BIOLOGICAL ASSESSMENT AND MOLECULAR MODELING OF NOVEL TACRINE-7-METHOXYTACRINE HETERODIMERS FOR ALZHEIMER DISEASE TREATMENT

J. Korabecny[1,2,*], S. Hamuľakova[3], K. Babkova[1], A. Horova[2], K. Musilek[4], K. Spilovska[2], J. Imrich[3], V. Opletalova[1], Z. Gazova[5], J. Hroudova[7], Z. Fisar[7], L. Gaľa[8], M. Valko[8], J. Kassa[2], K. Kuca[6,9]

[1]Department of Pharmaceutical Chemistry and Drug Control, Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic; [2]Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 0, Hradec Kralove, Czech Republic; [3]Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, P.J. Safarik University, Moyzesova 11, 041 67 Kosice, Slovak Republic; [4]Department of Chemistry, Faculty of Science, University of Hradec Kralove, Rokitanskeho 62, 500 05 Hradec Kralove, Czech Republic; [5]Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovak Republic; [6]Centre of Advanced Studies, Faculty of Military Health Sciences, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic; [7]Department of Psychiatry, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Ke Karlovu 11, 120 00 Prague 2, Czech Republic; [8]Faculty of Chemical and Food Technology, Slovak Technical University, Radlinska 9, 812 37 Bratislava, Slovak Republic; [9]University Hospital of Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Alzheimer disease (AD) is an irreversible, progressive neurodegenerative disorder associated with a global impairment of higher mental function that represents a deterioration of memory as the cardinal symptom. Early studies based on pharmacologic interference with cholinergic function suggested close relationship between acetylcholine-mediated neurotransmission and cognitive function. Acetylcholinesterase (AChE; EC 3.1.1.7) is an enzyme decomposing acetylcholine neurotransmitter to acetate group and choline. Inhibition of AChE by reversibly acting AChE inhibitors (AChEIs) is leading therapeutic approach in AD treatment leading to improvement of cognitive functions. Within our contribution we are presenting synthesis, biological evaluation and molecular modelling in the novel series of tacrine-7-methoxytacrine heterodimers as possible anti-AD agents. As resulted several candidates reached promising results comparable with those obtained for standard AD drug tacrine. Selected chemical structures will be in the future leading structures for in vivo toxicity studies.

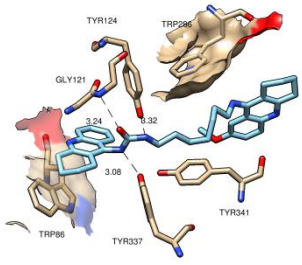

Fig. 1: Top-score docking pose of the most promising derivative depicted its putative hydrogen bonds formed with amino acid residues in the active-site gorge of the hAChE.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DUAL BINDING SITE CHOLINESTERASE INHIBITORS WITH BETA AMYLOID ANTIAGGREGATION ACTIVITY

N. Guzior[1,*], M. Bajda[1], M. Prinz[2], U. Holzgrabe[2], B. Malawska[1]

[1]Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, 30-688 Krakow, Medyczna 9, Poland
[2]Institute of Pharmacy and Food Chemistry, University of Wuerzburg D-97074 Wuerzburg, Am Hubland, Germany

The multi-target-directed ligand design strategy is an attractive approach in the development of novel effective drugs for treatment of disorders with complex pathological mechanisms such as Alzheimer's disease. In recent years many multifunctional compounds like dual binding site cholinesterase inhibitors have been described. Dual binding site inhibitors of cholinesterases influence acetylcholine hydrolysis reactions and peripheral anionic site dependent beta amyloid aggregation (1, 2).
As a continuation of our studies the new series of heterodimeric compounds were designed using molecular modeling. Several series of hybrid molecules bearing different substituted benzylamines linked by alkyl chain with heterocyclic isoindolino-1,3-dion were synthesized (Fig. 1) (2). Activity of the obtained compounds against cholinesterases (acetylcholinesterase from *electrophorus electricus* and butyrylcholinesterase from *horse serum*) was evaluated in spectrophotometric Ellman's assay. The new compounds were also examined against beta amyloid aggregation in fluorometric Thioflavine T test.
Among the novel series selective AChE inhibitors and both cholinesterases inhibitors were obtained. Their activity expressed as IC_{50} values ranged between 0.087 - 8.69 μM for AChE and 1.06 - 11.22 μM for BuChE. Some derivatives inhibited aggregation of beta amyloid in 22.72 – 39.60 % at 50 μM concentration.

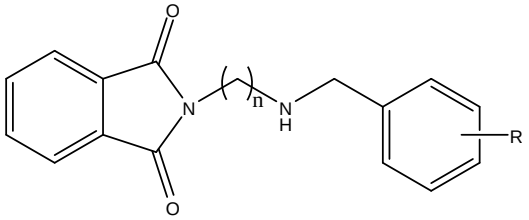

Fig. 1: Structures of obtained dual binding site cholinesterase inhibitors.

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STYRYLQUINOLINE DERIVATIVES POTENTIALLY USEFUL AS DIAGNOSTIC AND THERAPEUTIC AGENTS IN MISFOLDING DISEASES

M. Staderini[1,*], S. Aulic[3], M. Bartolini[4], H. Ngoc Ai Tran[3], V. G. Ruiz[2], D. I. Pérez[5], N. Cabezas[1], A. Martínez[5], M. A. Martín[2], V. Andrisano[4], G. Legname[3], J. C. Menéndez[1], M. L. Bolognesi[4]

[1]Departamento de Química Orgánica y Farmacéutica and [2]Sección Departamental de Química Analítica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain. [2]Statistical and Biological Physics Sector, Neurobiology Sector, SISSA-Unit, Italian Institute of Technology, 34014 Trieste, Italy. [3]Department of Pharmaceutical Sciences, Alma Mater Studiorum, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy. [5]Instituto de Química Médica-CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

The so-called "protein misfolding diseases" (PMDs) include a broad range of human disorders, such as Alzheimer's and prion diseases, characterized by a conformational change of normally expressed proteins that leads to the formation of fibrillar aggregates, causing neuronal damages (1). These amyloid aggregates can be considered both as a neuropathological hallmark and as a therapeutic target of these neurodegenerative disorders, and thus their detection represents a very important goal in the diagnostic field and has therapeutic implications as well. Several styryl derivatives have been employed to detect β-amyloid plaques (Aβ) (2) and some of them were also found to be active against prion replication (3). Therefore, it seemed conceivable that the styryl moiety is critical to recognize and interfere with amyloidogenic processes. With this in mind we generated a small library of styrylquinoline derivatives by linking the styryl moiety to a quinoline nucleus, the core of many anti-prion compounds and endowed with good pharmacokinetic properties (4). We explored the possibility of using our compounds as fluorescent probes for the optical imaging of amyloid plaques and therapeutic tools in Alzheimer's and prion diseases. We first studied their native fluorescence in different solvents presenting different polarities in order to mimic the protein environment. As regards the amino derivative, compound **1** (Fig.1), an emission maxima in the region of 600 nm was observed, where the potential interferences present in real biological samples can be avoided. We demonstrated that **1** is able to interact with both Aβ and prion fibrils and to inhibit Aβ self-aggregation and prion replication in the submicromolar range in a cellular context. Furthermore, it is not toxic and is able to cross the blood brain barrier *in vitro* (PAMPA test) (5).

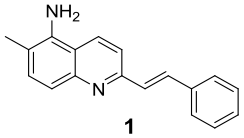

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Fig. 1: Structure of compound 1.

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DESIGN AND SYNTHESIS OF *trans*-DECAHYDROISOQUINOLINE DERIVATIVES AS NEW TRAMADOL-LIKE LIGANDS

R. Turnaturi[1,*], G. Ronsisvalle[1], L. Pasquinucci[1]

[1]Department of Drug Sciences, Medicinal Chemistry section. University of Catania, V. le A. Doria, 6 - 95125, Catania, Italy

Neuropathic pain is treated with many drug classes with a limited cost-benefit profile (1). Nucynta ER (extended release Tapentadol) is the newest compound to enter the neuropathic pain market and represents a clinical differentiation from other established treatment options due to its dual mode of action - agonism of the mu opioid receptor (MOR) and noradrenaline reuptake inhibition. Tapentadol is a derivative of Tramadol that are both clinically used analgesics. Multitarget ligands, with an opioid and non-opioid mechanism of action, showed favourable and safety clinical profile in neuropathic pain conditions requiring long-term management (2). So, with the aim to obtain compounds that maintained the mechanism of action of the parental ligands and showed an improved analgesic efficacy, some tramadol-like compounds have been designed and synthesized. Pharmacophoric features and their critical distances have been highlighted to identify a model that represented the interaction with MOR of these series of compounds. Thus, it has been synthesized a series of compounds containing the *trans*-decahydroisoquinoline nucleus (fig.1), in which two pharmacophoric elements of tramadol - the lateral chain and the basic nitrogen - are constrained in a cyclised structure.

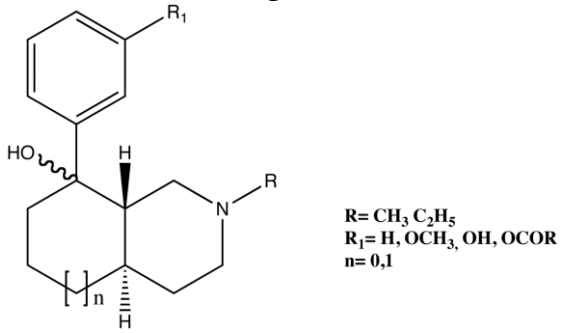

Fig. 1: Structure of tramadol-like compounds.

The constrained structure of *trans*-decahydroisoquinoline nucleus may exert a positive role by blocking the final compounds in a semi-rigid conformation that could enhance the MOR efficacy. In fact, the overall structure of these new compounds resembles the conformational characteristics of (+)-tramadol, the isomer with major affinities to MOR. To synthesize intermediates and final compounds conventional and (3) (4) microwaves-assisted methods were employed.

Synthesized derivatives will be pursued in *in vitro* and *ex vivo* studies to determine their affinities and activity versus MOR, DOR and KOR. Finally, in vivo antinociceptive profile in acute and chronic pain animal models could be investigated for most promising derivatives.

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POSTERS

MOLECULAR DOCKING AS A PREDICTIVE TOOL IN RATIONAL DESIGN OF SOFT CORTICOSTEROIDS DERIVED FROM PREDNISOLONE

V. Dobricic[1,*], N. Milenkovic[1], B. Markovic[1], S.Vladimirov[1], O. Cudina[1]

[1]Department of Pharmaceutical Chemistry, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

Soft corticosteroids are pharmacologically active compounds which undergo predictive metabolism to produce inactive and nontoxic metabolites. Soft corticosteroids are derived from cortienic acid which is a corticosteroid metabolite without anti-inflammatory activity (1). Cortienic acid (11β,17α-dihydroxy-3-oxo-androsta-1,4-dien-17β-carboxylic acid) was obtained by periodic oxidation from prednisolone according to the literature procedure (2). Several esters and amides of cortienic acid were selected for molecular docking calculations to find potentially active compounds. Molecular docking calculations were performed using Autodock v4.2 into the 3D structure of receptor for dexamethasone (pdb code:1m2z). 100 runs of Lamarckian genetic algorithm in AutoDock v4.2. software were performed in docking simulations for each structure. The structures were incorporated into 40x40x40 grid points receptor pocket, which was centered to the position of dexamethasone in crystallographic structure of the complex. AutoDock Tools was used for analysis of results. Binding energies of these structures were calculated and compared with the binding energy of prednisolone and cortienic acid obtained from prednisolone (Fig. 1). According to the results of the docking calculations, all of the tested structures have lower binding energies than cortienic acid. Binding energies of several structures are similar to or lower than the binding energy of prednisolone. This indicates that these structures might have anti-inflammatory activity. These structures will be synthesized and their anti-inflammatory activity will be tested *in vivo*.

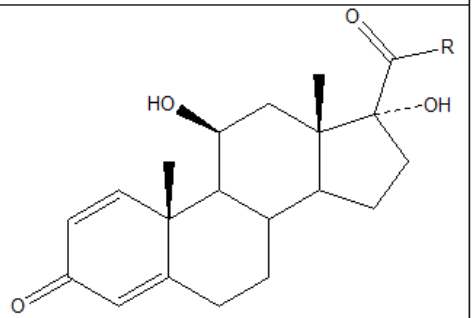
R	Binding energy (kcal/mol)	
$-NHCH_2COOCH_3$	-12.03	
$-NHCH_2\,CH_2Cl$	-11.46	
$-NH(CH)CH_3COOCH_3$	-11.17	
$-NH(CH)CH_3COOCH_2CH_3$	-11.34	
$-NH(CH)COOHCH_2CH_2COOCH_3$	-10.72	
$-O(CH)CH_3(CH)CH_3OH$	-12.47	
$-OCH_2CH_2CH_2Cl$	-11.84	
$-OCH_2CH_2OH$	-11.69	
$-OCH_2CH_2Cl$	-11.62	
-OH (cortienic acid)	-10.40	
$-CH_2OH$ (prednisolone)	-11.94	

Fig. 1: Binding energies of selected prednisolone derivatives.

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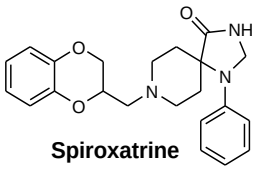
SYNTHESIS, STRUCTURAL CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SPIROXATRINE ANALOGS AS POTENTIAL NOCICEPTIN/ORPHANIN FQ RECEPTOR LIGANDS

S. Corrado[1,*], U. Battisti[1], C. Sorbi[1], D. Malfacini[2], G. Calò[2], L. Brasili[1], A. Tait[1]

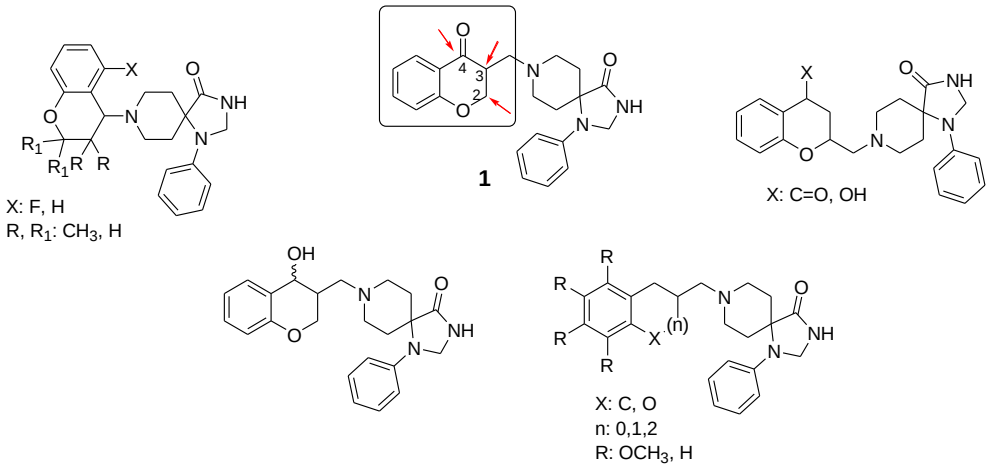
[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Modena and Reggio Emilia, via G. Campi 183, 41125, Modena, Italy
[2]Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, Via Fossato di Mortara 17-19, 44121, Ferrara, Italy

The nociceptin/Orphanin FQ (N/OFQ) peptide (NOP) receptor is a G protein-coupled receptor with a high degree of structural homology (~ 60%) to the classical opioid receptors μ, δ and κ. For this reason, NOP is considered the fourth member of the opioid receptors family, although it does not bind classical opioid ligands (1, 2).



Spiroxatrine

The interaction between NOP and its endogenous agonist N/OFQ plays a key role in pain transmission, among other biological functions. Therefore, this system opened a new option for the treatment of acute and chronic pain possibly by generating drugs with a lower side effect profile (2). The confirmed affinity towards NOP receptor of the α_2 adrenergic and 5-HT_{1A} partial agonist **spiroxatrine** (K_i= 127 nM) (3) has led us to the synthesis of a series of novel and optimized analogs based upon the spiropiperidine core. At first, we replaced the 1,4-benzodioxane moiety of spiroxatrine with the chroman-4-one moiety, to give the lead compound **1**. This lead has been modified in order to perform preliminary SAR studies, in particular positions 2, 3 and 4 of chroman core were explored as possible sites to connect spiropiperidine portion (Scheme 1). All synthesized compounds were evaluated at the recombinant human NOP using a calcium mobilization assay (4).



Scheme 1: Lead compound and example of some synthesized derivatives.

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MAGNOLIA EXTRACT, MAGNOLOL AND ITS METABOLITES: ACTIVATION OF CB_2 RECEPTORS AND BLOCKADE OF THE RELATED GPR55

A. Fuchs[1,*], V. Rempel[1], C. E. Müller[1]

[1]PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

The bark of *Magnolia officinalis* is widely used in the Chinese and Japanese traditional medicine. The fields of application vary from anxiety disorders, sleep problems and allergic diseases (1,2). Rodent studies have shown that preparations from *Magnolia* bark feature antidepressant, anxiolytic and anti-inflammatory effects. In these studies the neolignans magnolol, honokiol, methoxyhonokiol and 8,9-dihydroxydihydromagnolol were found to be responsible for the reported effects (3). Interestingly, these findings are similar to the effects reported for cannabinoid (CB) receptor ligands (4). In addition to this the neolignans show structural similarities to CB receptor ligands such as the highly potent synthetic CP55,940. Due to this, we investigated an extract of *Magnolia officinalis* bark, honokiol, magnolol and the main metabolites of magnolol (tetrahydromagnolol, 8,9-dihydroxydihydromagnolol) for their interaction with CB receptors and the related orphan receptor GPR55 (5). Tetrahydromagnolol could be identified as a potent selective partial agonist at CB_2 receptors and as an antagonist at GPR55. Tetrahydromagnolol also acts as a weak full agonist at CB_1. These findings indicate that the effects of *magnolia* bark are mediated via CB receptor activation.

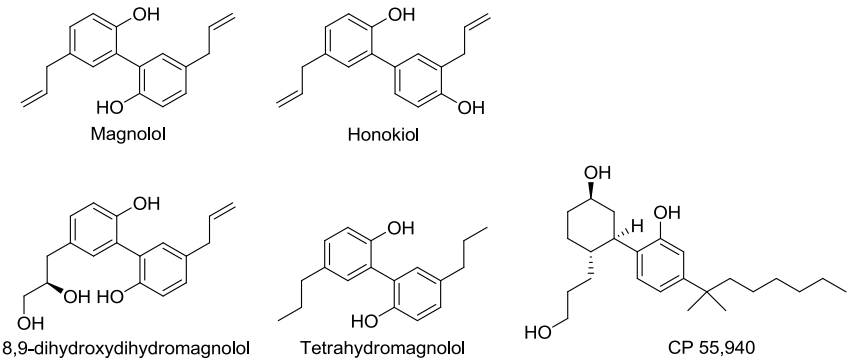

Fig. 1: Structures of *Magnolia* neolignans and metabolites and the synthetic CB receptor ligand CP55,940.

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INVESTIGATION OF BASIC MOIETIES CONFERRING SIGMA-2 RECEPTOR SELECTIVITY AND ANTIPROLIFERATIVE ACTIVITY

R. Marottoli[1,*], C. Abate[1], F. Berardi[1]

[1]Dipartimento Farmacochimico, Università degli Studi di Bari ALDO MORO, Via Orabona 4, I-70125 Bari, Italy

The interest for the σ receptors increased in the early 1990's when two subtypes, σ_1 and σ_2, were identified. The σ_2 receptor, that is the lesser known of the two subtypes, is overexpressed in a wide variety of cancer cell lines and a relationship between the proliferative status of tumors and the density of σ_2 receptors has been found. Several studies have shown that the density of σ_2 subtype is about 10-fold higher in proliferative than in quiescient tumor cells (1). Hence, these subtypes are proposed and studied as biomarkers for the diagnosis of solid tumor and their proliferative status. Besides, the interest for σ_2 receptors is related to potential tumor treatment since activation of σ_2 subtype causes antiproliferative effects in a number of tumor cell lines as well as in tumor animal models. Different experiments suggest that σ_2 receptor agonists induce cell death by both caspase-independent and caspase-dependent apoptosis. A promising agent for tumor therapy is the σ_2 receptor ligand 1'-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro-[isobenzofuran-(3*H*),4'-piperidine] (siramesine) that induces cell death through destabilization of lysosomes (2). Good results were also obtained with the high-affinity σ_2 receptor ligand 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperazine (PB28) that showed agonist σ_2 activity because it inhibited proliferation in several tumor cell lines (3). PB28 also proved to revert multidrug resistance (MDR) in cancer tumor cells treated with doxorubicin.
Therefore, with the purpose to better characterize the σ_2 protein and to identify new σ_2 ligands with potential antiproliferative activity, we synthesized two novel series of compounds using siramesine and PB28 as lead compounds. Basic moieties from PB28 series were inserted on siramesine hydrophobic portion and viceversa, basic moieties from siramesine series were inserted on PB28 hydrophobic portion. 4-Cyclohexylpiperidine, 4-cyclohexylpiperazine, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (from PB28 analogues) (4) and 1-(4-fuorophenyl)piperidine and spiro-isobenzofuran-1(3*H*),4'-piperidine (from siramesine analogues) were the basic moieties investigated. As for the indole-bearing compounds both 1-(4-fluorophenyl) substituted and unsubstituted indols were prepared for each basic moiety.
All prepared molecules will be evaluated for σ_2 affinity, selectivity versus σ_1 and antiproliferative activity. Also the capacity of these compounds to revert MDR in tumor cells will be tested with the aim of evaluating their potential use in anticancer therapy.

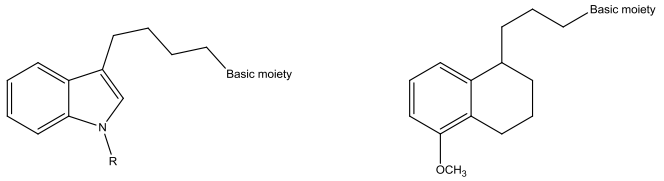

Fig. 1: General structure of new series of σ_2 ligands.

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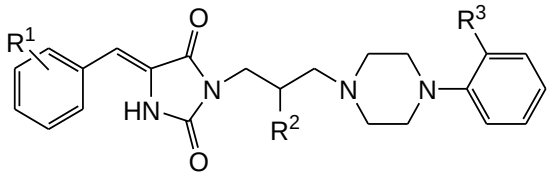
INFLUENCE OF THE STRUCTURE MODIFICATIONS AT ARYLIDENE PHENYLPIPERAZINE HYDANTOIN ON AFFINITY FOR ALPHA$_1$ – ADRENERGIC RECEPTORS

A. Dela[1,*], M. Dybała[2], A. Siwek[2], J. Handzlik[1], K. Kieć-Kononowicz[1]

[1]Dept. of Technology and Biotechnology of Drugs, [2]Dept. of Pharmacobiology Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków

Recently, α_1-ARs have been the subject of intensive research taking into account their potential role in arrythmia mechanisms, especially, in ischemic arrhythmia (1). The α_1-adrenoreceptors are the family of G-protein-coupled seven-transmembrane helix receptors. Studies on receptors binding have shown that the number of compounds with affinity for α_1-AR contain arylpiperazine moieties. In our previous research, several N1-phenylpiperazine derivatives of diphenyl hydantoin were obtained (2,3). The compounds have shown affinity for α_1–ARs in submicromolar range (K_i).

In our recent studies, new N3-phenylpiperazine derivatives of 5-arylidenehydantoin were synthesized. They have shown high affinity for α_1-ARs. The obtained structures contain arylidene hydantoin moieties with methoxy substituent(s), hydroxypropyl or propyl linker and phenylpiperazine (non substituted or substituted with methoxy or ethoxy substituent). General structure of the compounds is presented in **Fig. 1**:



R^1: 3,4-diOCH$_3$
R^2: H, OH
R^3: OCH$_3$, OC$_2$H$_5$

Chemical modifications were focused on the introduction of one-, two- or three methoxy substituents at arylidene ring of the lead and hydroxypropyl or propyl chain between arylidene hydantoin and phenylpiperazine moieties. The new compounds were obtained within four-step synthesis: (1) Knoevenagel condensation, (2) Mitsunobu reaction, (3) N-alkylation under microwave irradiation and (4) conversion of the obtained basic derivatives into the corresponding hydrochloric form. The new hydantoin derivatives were evaluated on their affinity for α_1-adrenoreceptors in radioligand binding assay using [^{3}H]prazosin as selective radioligand. The assay indicated promising affinities for α_1-AR in whole tested population however better for compounds with propyl linker (Ki = 11,9 – 40,2 nM) than those with hydroxypropyl one (Ki = 23.1 nM – 8.7 μM). SAR-sudies indicated a profitable influence of alkoxyl substituents at both, aryl- and arylidene fragment and propyl linker on α_1-AR affinities. In next studies we should synthesize the new compounds with longer chain between arylidenehydantoin and phenylpiperazine. We should also consider introduction of (mono or di)chloro substituent in phenylpiperazine and arylidenehydantoin moieties.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL DUAL ANTITHROMBOTIC AGENTS – INHIBITORS OF FACTOR Xa AND ANTAGONISTS OF GPIIb/IIIa

U. Trstenjak[1,*], J. Ilaš[1], D. Kikelj[1]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

Cardiovascular diseases, such as myocardial infarction, stroke, unstable angina pectoris and pulmonary embolism, are a major cause of mortality in developed world (1). Based on previous work in our group that led to promising agents combining thrombin inhibitory and glycoprotein IIb/IIIa receptor antagonistic activity (3), we decided to design novel dual antithrombotic agents combining factor Xa inhibitory and glycoprotein IIb/IIIa receptor antagonistic activity. Known crystal structures of factor Xa in complex with rivaroxaban, its potent direct inhibitor (4) and GP IIb/IIIa cocrystallized with its antagonist tirofiban (5) were used for docking of virtually designed molecules combining pharmacophores of rivaroxaban and RGD sequence (anionic and basic center in appropriate distance), which is responsible for recognition and binding of various adhesive endogenic protein ligands to GP IIb/IIIa. According to results of docking and synthetic options we prepared novel molecules consisting of various fragments bearing carboxylic acid moiety as anionic center and fragments bearing basic center attached to opposite sides of rivaroxaban central core (Fig. 1). Biological evaluation of given compounds including determination of K_i on factor Xa and IC_{50} on GP IIb/IIIa, as well as study of selectivity on serine proteases by determination of K_i on thrombin and trypsin gained insight into structure-activity relationships that will be used in further optimisations towards novel dual antithrombotic agents.

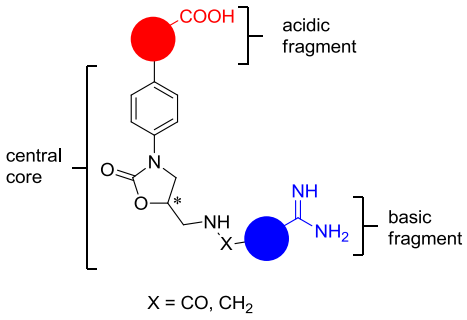

Fig. 1: Structure of novel dual antithrombotic agents – inhibitors of factor Xa and antagonists of GPIIb/IIIa.

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SYNTHESIS AND EVALUATION OF NOVEL NORBORMIDE DERIVATIVES AS VASOACTIVE COMPOUNDS

G. Ribaudo[1,*], E. Costa[1], R. Pasquale[1], B. Hopkins[2], S. Bova[1], G. Zagotto[1]

[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy
[2]Landcare Res., Canterbury Agr. & Sci. Ctr., Lincoln, New Zealand

Norbormide (NRB) has been known as a specie-specific vasoconstrictor rat toxicant (LD_{50} 5-10 mg/kg) introduced in the '60s as a result of a screening for novel potential anti-inflammatory and anticholinergic compounds (1). The vasoconstrictor activity is only limited to peripheral small caliber arteries and veins, whilst big caliber vessels (aorta) are not involved. The compound does not show any remarkable effect on other mammifers and, in particular, humans. Despite norbormide has been introduced over forty years ago, a fully explicative mechanism of action has never been reported; nonetheless, a phospholipase C activation seems to be involved (2). The aim of the project was to synthesize and evaluate novel analogues, and to identify the pharmacophoric groups in order to put the bases for a ligand-based compound design and to better understand the mechanism of action. Ideally, the identification of this pathway could represent a source of inspiration for a new target in cardiovascular medicinal chemistry. From a structural point of view, norbormide is a rather complex norbornenedicarboxyimide and it has always been used as a mixture of eight isomers. The molecular structure encloses three variables: the orientation of the C_7-C_8 double bond, the configuration of the asymmetric alcoholic carbon in *alpha* to C_5 and the *endo* or *eso* form of the imide (C_2-C_3). The *endo* isomers are known to produce more vasoconstriction (3). A first set of analogues (SET1) was obtained through the reaction of anhydridic precursor with amines (the reaction was carried out at different conditions of solvent and temperature). Unfortunately, none of such compounds showed a significant vasoconstrictor activity. Later, a second set of compounds (SET2) was synthesized with a positive charge in the molecule through the alkylation of the pyridine linked to the C_8. The introduction of these groups and the charge were intended to interfere with the spatial orientation of the aromatic system. The quaternary nitrogen could also modify the degree of absorption (if any) through the cell membrane. This set of analogues looked more promising as long as we identified some compounds with a peripheral vasoconstrictor activity comparable to norbormide.

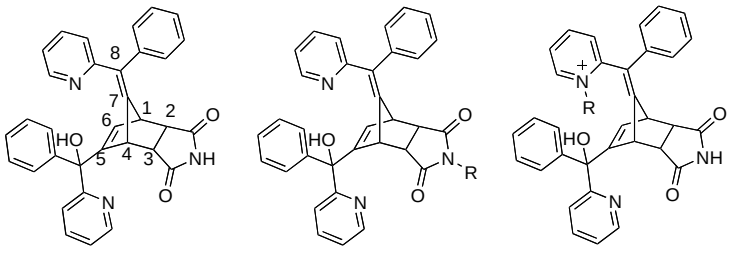

Fig. 1: Chemical structures of the cited compounds.

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A NEW APPROACH TO BIOACTIVE CARBOLINES (1)

P. Sánchez-Alonso[1,*], R. Alajarín[1], J. Álvarez-Builla[1], J. J. Vaquero[1]

[1]University of Alcalá, Dept. Organic Chemistry, Ctra. Madrid-Barcelona km 33.6, 28871-Alcalá de Henares, Madrid, Spain

Carbolines, a family of heterocyclic systems widely spread in nature, and specially β-carbolines (2), have been reported to show a variety of interesting biological properties such as antiviral and antitumor agents, inhibition of topoisomerase II, anxiolytic, anti-inflammatory, CNS-stimulating activity, acetylcholinesterase (ACHE) inhibition, antioxidant, anti-HIV, antibacterial, antiparasite, antidiabetic or 5-HT antagonist activity. The synthetic access to this class of compounds have been of interest to many researches in order to obtain carboline-based natural and synthetic compounds or modified analogues with improved or new biological activities. For instance, compounds **I** and **II** have been reported as anti-Leishmanial compounds (3) and compound **III** shows inhibition on fatty acid binding protein (FABP) (4) (Fig. 1), two fields of interest to our group.

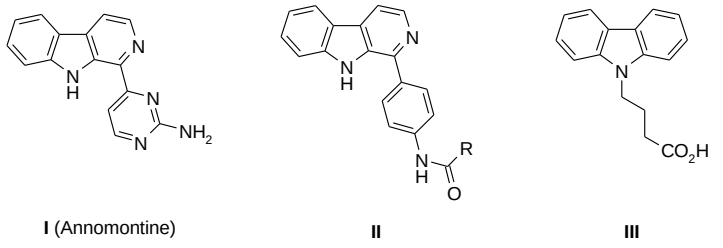

Fig. 1: Natural and unnatural β-carboline-type compounds with anti-Leishmanial activity and FABP inhibition.

In our search for new compounds with biological activity we have contributed to the state-of-the-art heterocyclic synthesis using the chemistry of TosMIC (5). In this regard, we are exploring the use of TosMIC-based cyclization reactions for the synthesis of six-membered heterocycles. In this communication, we wish to report the synthesis of compounds of general structure **IV** and **V** and the results obtained when heterocyclization reactions leading to β- and γ-carbolines were studied (Fig. 2).

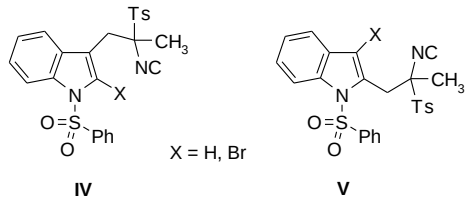

Fig. 2: Isocyanides explored in heterocyclization reactions.

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(1) This work was financially supported by Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo (RD006/0016/0016) and Ministerio de Economía y Competividad (CTQ2011-24715).

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A VERY SHORT SYNTHESIS OF BRIDGED BENZODIAZOCINES WITH A BISPIDINE-LIKE CAVITY

P. Ribelles Torres[1,*], D. K. Becerra Córdoba[2], M. T. Ramos García[1], J. C. Menéndez Ramos[1]

[1]Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal S/N, 28040, Madrid, Spain
[2]Departamento de Química, Facultad de Ciencias Naturales y Exactas, Universidad del Valle, Edificio 320 - Ciudadela Universitaria Meléndez, Cali, Colombia

Sudden cardiac death (SCD) is a leading cause of mortality in which ventricular arrhythmias are believed to play a major role. Prevention and control of ventricular tachycardias (VT)/ventricular fibrillation (VF) have a very significant therapeutic importance, and for this reason a large number of new antiarrhythmic agents (AAA) have been prepared and studied in the last decade. The so-called Human Ether-a-go-go-Related Gene (hERG) potassium channels conduct the rapid component of the delayed rectifier potassium current, IKr, which is crucial for repolarization of cardiac action potentials. Moderate hERG blockade leads to a beneficial class III antiarrhythmic effect (1), although an excessive reduction in hERG currents can lead to hereditary or acquired long QT syndromes characterized by an increased risk for "torsade de pointes" arrhythmias and sudden death. Suitably modified bispidine and oxabispidine derivatives have overcome this problem and are one of the most promising drug candidates acting on the hERG target (2,3).
We report here a two-step protocol (Fig. 1) that affords diazocine derivatives with a bispidine-like cavity (compounds **2**). Moreover, the presence of an aromatic ring fused to the nitrogenated bicycle allows to modulate the size and characteristics of the cavity. The starting materials **1** were obtained from a diastereoselective Povarov-like reaction between acyl imines and α,β-unsaturated hydrazones catalysed by $InCl_3$ (4) and a subsequent domino reduction-cyclization-reduction process in the presence of $NaCNBH_3$ afforded compounds **2** with full diastereoselectivity.

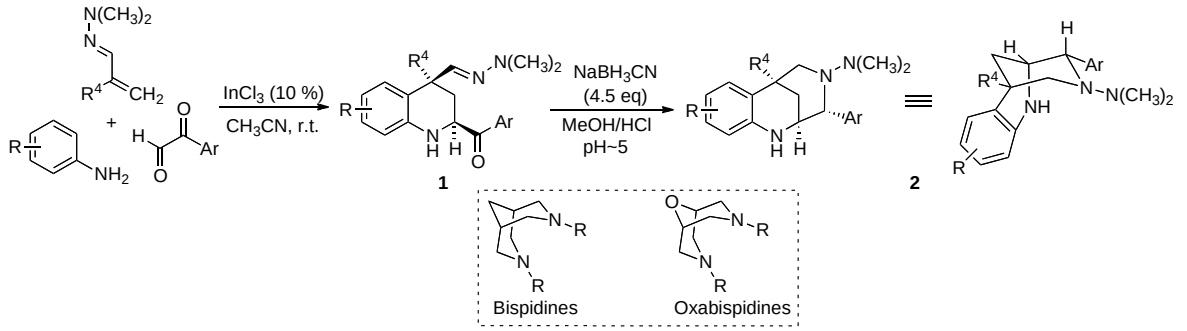

Fig. 1. Diazocine synthesis starting from Povarov-like products obtained from α,β-unsaturated hydrazones as dienophiles.

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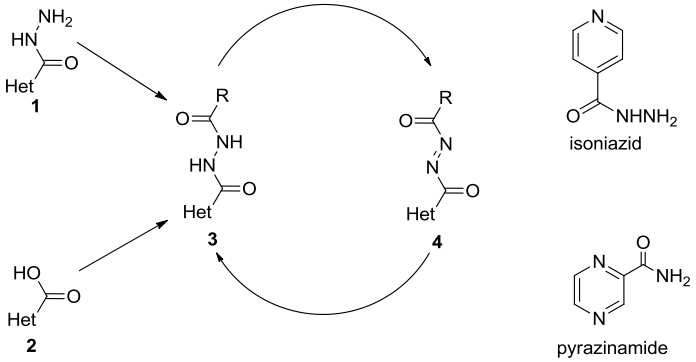
HETEROCYCLIC HYDRAZIDES AS SYNTHETIC TARGETS AND THEIR FURTHER TRANSFORMATIONS

J. Vajs[1,*], M. Kočevar[1], S. Polanc[1]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, 1000 Ljubljana, Slovenia

According to the World Health Organisation, one-third of the world population is currently infected with tuberculosis. Due to arising resistance to the established medicines, which are used to treat this disease and concomitant HIV infection which occurs quite often, it is urgent to develop new agents which will treat this lethal disease more efficiently.
From commercially available hydrazides **1** and carboxylic acids **2** we have prepared new hydrazides **3**, analogues of isoniazid and pyrazinamide, which are currently the most commonly used drugs for the treatment of tuberculosis. Furthermore, we have evaluated the scope and limitation of several oxidizing agents for the transformation of hydrazides **3** to diazenes **4** (1) and found the combination of NBS and pyridine to be the most successful. With this combination, we oxidised previously synthesized hydrazides **3** to diazenes **4**. The diazenes **4** prepared according to this procedure (with nearly quantitative yields) turned out to be effective oxidants of thiols to disulfides. This means that they could be used for the oxidation of glutathione (GSH) to the corresponding disulfide (GSSG); namely, the ratio of GSH to GSSG in all living cells effects several physiologically important biochemical processes (2), thus most probably the obtained diazenes **4** would exhibit important biological activity.



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AN INFLUENCE OF ARYLIDENEIMIDAZOLONES ON MULTIDRUG RESISTANCE MECHANISMS IN GRAM-NEGATIVE BACTERIA

E. Otrębska[1,*], J. Handzlik[1], B. Mastek[1], S. Alibert-Franco[2], J. Chevallier[2], J. M. Pagés[2], K. Kieć-Kononowicz[1]

[1]Faculty of Pharmacy, Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, ul. Medyczna 9, 30-688 Cracow, Poland
[2]Facultés de Médecine et de Pharmacie, Transporteurs Membranaires, Chimiorésistance et Drug-Design, UMR-MD1, 27 Bd Jean Moulin, 13385 Marseille cedex 05, France

Today we assist to the re-emergence of infectious diseases involved in more than 15 million deaths each year. One of the reasons for this situation is the increasing resistance of pathogenic bacteria to available antibiotics. The multidrug resistance (MDR) seriously limits treatment of various bacterial diseases (1). Microbial efflux pumps play a key role in MDR strains (2). One of the ways to combat MDR is search for new chemical compounds that are able to inhibit protein pump system responsible for drug efflux, consequently increase antibiotic concentration in a target cell (3). In this context, a series of new arylideneimidazolone derivatives (Fig. 1) were evaluated on their efflux pumps inhibition (EPIs) properties in microbiological assays in strains of *E. aerogenes* with different expression of AcrAB-TolC (EA294, EA289 and CM64).

R= *p*-chlorophenyl, 2-thiophenyl, 3-thiophenyl, β-naphthyl, 2-fluorenyl

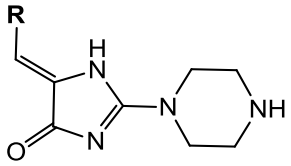

Fig. 1. Structures of the tested compounds.

Direct antibacterial activity of the compounds, their influence on minimal inhibitory concentration (MIC) of antibiotics as well as their cooperation with the antibiotics (isobolograms) was determined. The real-time efflux test to identify the compounds that act on efflux in EA289 strain by blocking the expelling of fluorescent dye were carried out, too. Most of the compounds displayed ability to increase efficacy of antibiotics in strains over-producing AcrAB-TolC. The compounds cooperated with chloramphenicol, doxycycline and nalidixic acid in synergistic way, but additive cooperation was observed in the case of erythromycin. The highest EPIs properties in the real-time efflux assay were observed for the 5-fluorenemethylidene derivative.

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STRUCTURE-BASED DISCOVERY OF NOVEL INHIBITORS OF HUMAN TOPOISOMERASE IIα

B. Merkač[1,*], A. Perdih[1], M. Brvar[1], T. Solmajer[1]

[1]National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

DNA topoisomerases comprise a large family of enzymes that catalyse topological changes in DNA. These enzymes perform their functions by creating transient double-stranded breaks in the DNA molecule in order to allow another DNA molecule pass through the break. Due to their role in inducing topological changes, DNA topoisomerases represent one of the important targets for cancer chemotherapy (1). Human topoisomerase IIα is a homodimer macromolecule, which consists of three domains and has a close homology with bacterial the DNA gyrase (2). Topoisomerase II targeting agents are classified into two major groups that differ in their mechanism of action. First, a more established group of molecules are named poisons, due to their role in stabilization of the covalent cleavage complex and conversion of this enzyme into a cellular toxin which is lethal to normal cells. The second group are designated as catalytic inhibitors, and differ from poisons since they do not stabilize the covalent cleavage complex, but interfere in other steps of the described catalytic cycle (3,4).
DNA topoisomerase II is a well-established and validated target for development of novel antitumor agents. The aim of our research was to identify novel inhibitors that bind to human ATPase domain and thus blocking binding of ATP molecule. Starting from available structural information we identified a novel series of purine-based inhibitors of topoisomerase IIα by implementing structure-based design procedure (5). This novel class of DNA topoisomerase IIα inhibitors was further investigated by surface plasmonic resonance.

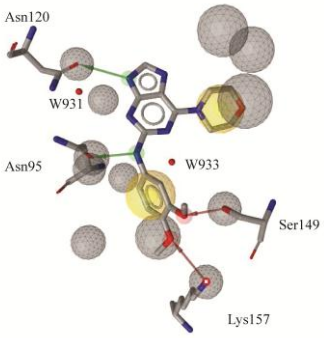

Fig. 1: Conformation of purine-based inhibitor with micromolar activity docked into ATPase domain of human topoisomerase IIα.

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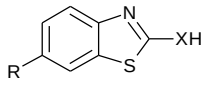
DESIGN, SYNTHESIS AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF 1,3-BENZOTHIAZOLES

I. Defrenza[1,*], M. Muraglia[1], A. Catalano[1], F. Corbo[1], C. Franchini[1]

[1]Medicinal-Chemistry Department, University of Bari "Aldo Moro", Via Orabona n. 4, 70125, Bari, Italy

One of the major problems we are facing today in the context of infectious diseases is the relentless increase and spread of antimicrobial resistance. Thus, there is an urgent need for new and different antimicrobial drugs. Many heterocyclic nuclei, such as 1,3,4-thiadiazole, benzimidazole, 1,3,5-triazine, and benzothiazole have been recently reviewed as antimicrobial agents (1). Our attention was focused to the benzothiazole nucleus. Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor, schistosomicidal, anti-inflammatory, anticonvulsants, antidiabetic, antipsychotic, diuretic and antimicrobial (2). In the past, our research group was interested in a series of 2-mercapto-1,3-benzothiazole derivatives (Fig. 1, X = S) showing antibacterial activity against Gram positive and negative (3). In this work, we thought to evaluate some of these compounds for their *in vitro* antifungal activity against *Candida* spp. Then, in order to improve SAR studies on activity against either Gram positive and negative bacteria or fungi species such as *Candida* spp of 1,3-benzothiazoles, we decided to investigate the isosteric relationship between the 2-mercapto and 2-amino function (X = NH) and the effects of substitutions at the C6 position with halogen, alkyl and aryloxy moieties. Results of this study will be presented.



X = NH, S

R = halogen, alkyl, aryloxy

Fig. 1

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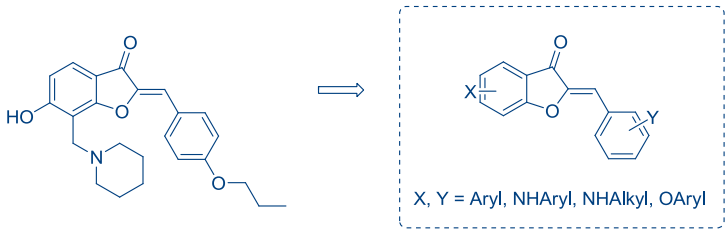
SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL AURONE DERIVATIVES AS POTENTIAL ANTIMALARIAL AGENTS

M. P. Carrasco[1,*], D. J. V. A. dos Santos[1], J. Gut[2], P. J. Rosenthal[2], R. Moreira[1]

[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[2]Department of Medicine, San Francisco General Hospital, University of California at San Francisco, CA 94143, USA

Malaria is responsible for causing an estimated 225 million clinical cases and one million deaths annually (1) being drug resistance to currently established antimalarial drugs such as chloroquine (CQ) a major problem of concern. Therefore, novel and innovative inhibitors active against *Plasmodium falciparum*, which produces the most aggressive form of malaria, are urgently required to develop new treatments able to fight malaria (2).
Aurones are secondary metabolites belonging to the flavonoids family and their antimalarial activity was already recognized (3). More recently, it was shown that the mechanism of action of this family is most likely a CQ-like action, i.e., by inhibiting the hemozoin formation inside the acidic digestive vacuole of the parasite (4). Degradation of hemoglobin by malaria parasite proteases causes the release of ferriprotoporphyrin IX (FPIX), which is detoxified by crystallization to hemozoin in the digestive vacuole. CQ and related antimalarial drugs, bind to FPIX via π-π stacking of the aromatic moiety with the porphyrin ring, thus inhibiting detoxification (5).
In an attempt to obtain new potent antimalarial agents and explore the chemical space around this scaffold, a library of novel aurone derivatives was synthesized by introducing an additional aromatic moiety through Suzuki–Miyaura and Buchwald–Hartwig cross-coupling reactions. The synthetic procedures and some preliminary results will be presented.



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DESIGN AND SYNTHESIS OF β-HYDROXY-β-ARYLPROPANOIC ACID

J. Savić[1,*], J. Brborić[1], S. Dilber[2], S. Vladimirov[1]

[1]University of Belgrade-Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Vojvode Stepe 450, 11221 Belgrade, Serbia
[2]University of Belgrade-Faculty of Pharmacy, Department of Organic Chemistry, Vojvode Stepe 450, 11221 Belgrade, Serbia

Non-steroidal anti-inflammatory agents are numerous, broadly used and can be procured as OTC drugs. Nevertheless, search for new non-steroidal anti-inflammatory agents is continuing. The main motive is to find compound that would selectively inhibit inducible form of cyclooxygenase (COX-2), but without effect on constitutive form (COX-1) (1). If this selectivity concept is achieved, adverse effect on gastric mucosa would be avoided. Based on our previous work (2), we decided to design and synthesize β-hydroxy-β-arylpropanoic acid that will have a polar nitro group in its structure (3-hydroxy-3-(4-nitrophenyl)-3-phenylpropanoic acid), which should contribute to the selectivity of the compound towards COX-2. The compound is structurally similar to ibuprofen, so in order to compare binding energies of our compound and ibuprofen to COX-1 and COX-2 docking calculations were performed using Autodock v4.0.1 into the 3D structure of the catalytic site of COX-2 enzyme (pdb code: 1cx2) and COX-1 enzyme (pdb code: 1eqg).
The structure of compound was generated using the ChemOffice v7.0 Ultra software package and have been MM2 optimized. Each docking experiment consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations. Other parameters were left to their default values. The search was conducted in a grid of 40 points per dimension and a step size of 0.375 centred on the binding site of enzyme. Results show improved binding energies of examined compound compared to ibuprofen.
The compound was synthesized using modification of Reformatsky reaction that has already been reported (3). Additional modification considering higher temperature (65-69°C) was introduced. Higher temperature significantly reduces duration time of the reaction and increases yield and purity of the synthesized compound. The compound was fully characterized using NMR, IR, MS-TOF and it is ready for biological testing.

Compound	Binding energy kcal/mol	
	COX-1	COX-2
(3-hydroxy-3-(4-nitrophenyl)-3-phenylpropanoic acid	-6,74	-6,21
ibuprofen	-8,56	-8,49

Table 1: Binding energies of the synthesized compound and ibuprofen.

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FLEXIBLE MOLECULES INCORPORATING A PIPERAZINE MOIETY AS POTENTIAL VOLTAGE-GATED SODIUM CHANNEL MODULATORS BASED ON MARINE SPONGE ALKALOIDS

M. Jukič[1,*], M. Anderluh[1]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

Interest in natural compounds as novel leads is rekindled in academia and industry over the past years. Namely, natural compounds in general cover a vast chemical space, often with favourable pharmacokinetic profiles, which makes these molecules an attractive target for further development (1,2,3).

Marine organisms present an under-exploited source, and among them sponges and their sponge-symbiotic microorganisms produce a variety of natural products with selective binding to biological targets (cytotoxins, antibiotics, antivirals, anti-inflammatory compounds, antifouling agents, etc.) (4). Sponges of the genus *Agelas* have been shown to produce compounds that modulate activity on voltage-gated sodium ion channels (5). Our goal was to design, synthesize and evaluate the activity of *Agelas* sponge alkaloid analogues. To examine in depth the pharmacophore model of oroidin, hymenidin and clathrodin marine alkaloids, we have synthesized a focused library of compounds with a piperazine central linker that replaces the 3-aminoprop-1-enyl moiety in the native compounds (Figure 1). 2-Aminoimidazole basic centre was joined via the afore-mentioned flexible linker to various aromatic or heteroaromatic synthons to afford final compounds. Our library of synthetically accessible, simple and flexible analogues of parent alkaloids is a suitable tool to examine the effect of terminal basic-aromatic centres on the activity of parent compounds. Electrophysiological measurements of synthesized analogs on Na_V 1.1–1.8 channel subtypes are still in a phase of biological evaluation but preliminary studies on Na_V 1.3 channel show that (4-((2-amino-1*H*-imidazol-5-yl)methyl)piperazin-1-yl)(1*H*-indol-3-yl)methanone molecule, where indole-3-carboxylate replaces the pyrrole terminal moiety, exhibits an IC_{50} of 19 μM, which is a reasonable improvement over the native compounds and offers a proof of concept that further modification of natural leads may lead to more potent synthetic analogues.

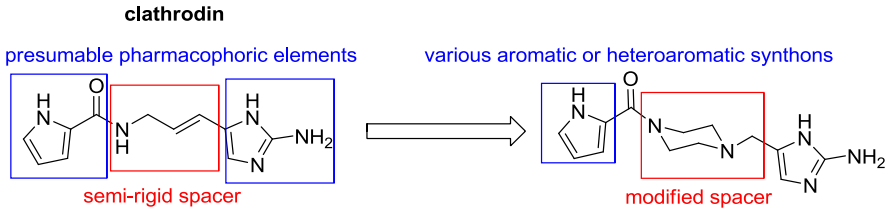

Fig. 1: Flexible analogue incorporating a piperazine-linker based on clathrodin, an *Agelas* marine sponge alkaloid.

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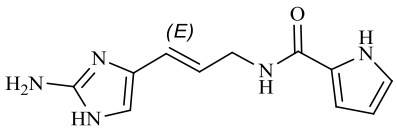
CLATHRODIN AS A VOLTAGE-GATED SODIUM CHANNEL BLOCKER

A. Žula[1], J. Ilaš[1], D. Kikelj[1]

[1]Faculty of Pharmacy, Department of Medicinal Chemistry, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

The sea is the oldest and the most extensive ecosystem on Earth, where marine plants and other organisms develop a number of compounds for the adaptation and struggle for existence with other species. One such compound is also clathrodin. Clathrodin is alkaloid first isolated from sponges *Agelas clathrodes* in 1991 and is an important member of a wide pyrrole-2-aminoimidazole (P-2-AI) family, because it represents the biosynthetic building block for the P-2-AI family of marine alkaloids (1). In biological tests clathrodin exhibited a broad spectrum of bioactivity, where the blockade of voltage-gated sodium ion channels was the most expressed. In the test system on cells isolated from the sympathetic ganglia of chicken embryos was for clathrodin revealed a blockade of voltage-gated sodium ion channels in the micromolar range (2). Quite a few synthetic procedures have already been published for the preparation of the clathrodin (3,4). In the context of the research work, we carried out with the classical principles of the organic chemistry a new, green and simple total synthesis of clathrodin, which has an ability to produce a set of clathrodin derivatives. The synthesis started from pyridine, which was acylated with benzyl chloroformate to form *N*-Cbz-pyridinium salt. This pyridinium salt intermediate was reduced with sodium borohydride ($NaBH_4$) to afford *N*-Cbz-1,2-dihydropyridine **1**. The preparation of this 1,2-dihydropyridine was hampered by a regioselectivity problem leading to formation of the 1,4-dihydropyridine regioisomer **2** (20% yield). These two compounds were successfully seperated by column chromatography. *N*-Cbz-1,2-dihydropyridine in the next step reacted with Boc-guanidine in the presence of 1 equiv of bromine to obtain the bycyclic compound **3** (39% yield) and its regioisomer **4** (19% yield). Bycyclic compounds **3** and **4** were treated in the presence of 6N HCl in methanol to directly obtain compound **5**. Acidic conditions lead to isomerization from *Z* to *E* of double bond of compound **5**. In the next step, compound **5** was acylated with pyrrole-2-carboxylic acid through standard coupling procedure with TBTU/NMM in DMF to obtain clathrodin (Fig. 1). We managed to prepare marine bioactive compound - clathrodin in an amount needed for structural characterization, biological studies and for further modification. Prepared clathrodin was sent for biological screening, which is currently underway, and findings will be reported in due course. In order to elaborate a broad structure-activity relationship analysis (SAR) will be with the principles of the ligand-based drug design a new library of clathrodin analogues prepared.



CLATHRODIN

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STUDY OF BIOLOGICAL AND HYDROPHOBIC PROPERTIES OF *N*-BENZYLPYRAZINE-2-CARBOXAMIDES

B. Servusová[1,]*, M. Doležal[1], M. Klementová[1], V. Kubíček[2] and K. Kralová[3]

[1]Faculty of Pharmacy, Department of Pharmaceutical Chemistry and Drug Control, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[2]Faculty of Pharmacy, Department of Biophysics and Physical Chemistry, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[3]Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska dolina Ch-2, 84215 Bratislava, Slovakia

The main determinants of a compound's biological activity and its physiological effects are relevant structure and physicochemical properties of the compound. Lipophilicity, one of the most important physicochemical properties of the compound, which seems to be a key factor related to the cell transmembrane transport and other biological processes, can either be determined experimentally or predicted by means of commercially available programmes and is usually expressed by the logarithm of the partition coefficient between *n*-octanol and water (log *P*) (1). Calculated values of log *P* are dependent on the size of the compound and the algorithm used for calculation and may not be reliable, *e.g.* possible intramolecular interactions affecting lipophilicity must be taken in account (2). We used pyrazinamide (PZA) as a model structure for substances referred in this research project. A series of substituted *N*-benzylpyrazine-2-carboxamides was synthesized by aminolysis of substituted pyrazinoylchlorides with corresponding benzylamines. Substitution of aromatic ring was chosen on the experience with analogously substituted *N*-phenylpyrazine-2-carboxamides, which have shown interesting antimycobacterial activity in comparison with PZA (3). The prepared compounds were evaluated for their *in vitro* antimycobacterial activity and ability to inhibit photosynthetic electron transport (PET). We focused on a comparison between calculated and experimentally determined lipophilicity parameters of the studied compounds. Log *P* values of the compounds were calculated by means of programmes. However, the obtained results differed, so we tried to find a more reliable method for the evaluation of lipophilicity. We decided to verify the results experimentally by means of the RP-HPLC determination of capacity factors *k* with subsequent calculation of log *k*. The values of PET inhibition were compared with measured log *k*. The results will be discussed.

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THE DESIGN AND SYNTHESIS OF NOVEL TRICYCLIC NUCLEOSIDES AS POTENTIAL ANTIPROLIFERATIVE AGENTS

K. Panagiota[1,*], L. Nikolaos[1], K. Ioannis[1], M. Panagiotis[1], P. Nicole[1]

[1]Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771

The nucleotides are the structural units of nucleic acids and play a crucial role in many metabolic processes. The study of their structural analogues is an important area of research within biological sciences, and numerous derivatives possess therapeutic interest, mainly as antiviral and anticancer drugs. The plant hormones cytokinins, which chemically are adenine analogues, promote cell division and differentiation. Their physiological role has prompted the development of compounds that might repair dysfunctions of cell division and differentiation in animal cells, as in the case of cancer cells. Furthermore their ribonucleotide derivatives, natural and synthetic, inhibit proliferation of cancer cells through selective activation of apoptosis and simultaneous blockade of the transition of G1 / S cell cycle. Based on the above mentioned considerations, a number of non-classical adenosine derivatives have been prepared in order to study their potential antiproliferative activity. The new molecules bear a tricyclic structure mimicking the purine system and possess alkylamino substituents which are present into active cytokinins. The derivatives were synthesized using as starting material 2,6-diaminopyridine which upon acetylation, nitration, selective deacetylation and reduction of the nitro group revealed an intermediate o-diamine. This compound was converted to the corresponding imidazolpyridine which was then subjected to nitration, reduction of the nitro group and reaction with glyoxal to provide imidazo[4',5':5.6]pyrido[2,3-*b*]pyrazine. Glycosylation of this molecule gave the two possible regio-isomers. The major isomer was converted to the 9-chloro derivative which was used for the preparation of the 9-aminosubstituted analogues.

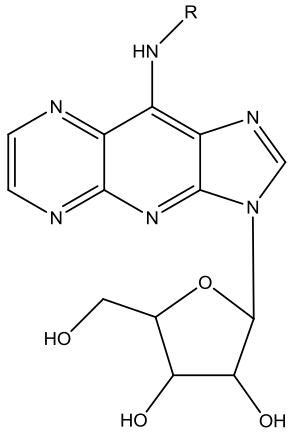


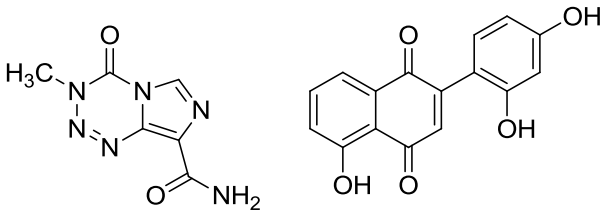
Fig. 1: General structure of the novel tricyclic nucleosides.

8-HYDROXYNAPHTALENE-1,4-DIONE DERIVATIVE AS NOVEL COMPOUND FOR GLIOMA TREATMENT

E. Costa[1,*], G. Ribaudo[1], G. Zagotto[1]

[1]Department of Pharmaceutical and Pharmacological Science, University of Padova, Via Marzolo 5, 35131 Padova, Italy

Malignant gliomas affect the central nervous system (CNS) and are the most frequent subtype of primary brain tumors (1), with high resistance to the standard cancer therapies.
At present the approved approach consists of neurosurgical resection, followed by chemotherapy with an alkylating agent, such as temozolomide (TMZ), in combination with radiotherapy (2).
In previous years, oncogenesis has been increasingly associated with an altered protein kinases expression (3). In particular, it was noticed that the interference with the protein kinase A pathway induced modulations in the proliferation and cell death of glioma cells (4).
This study investigated novel potential species for malignant glioma treatment. Preliminary screening of a small library of compounds from in house database showed that 2-(2,4-dihydroxyphenyl)-8-hydroxy-1,4-naphthoquinone (C1) emerged as a promising therapeutic lead. The cytotoxic effect of C1 was evaluated in the immortalized human glioma cell line GLI36 and in primary human glioblastoma cell culture, and compared with the effect induced by TMZ. In vitro pharmacological testing showed a remarkable increase of apoptosis on both human glioma cell line GLI36 and in primary human glioblastoma cell culture, when compared with TMZ.
Aiming for the discovery of novel therapeutic compounds which could significantly improve contemporary glioma chemotherapy, these preliminary results prompted the preparation of novel derivatives, whose testing is currently being carried out. In addition, future cytotoxicity assays on different cell lines will be considered, thus giving significant and comprehensive data to better understand the cytotoxic effect of such compounds.



TMZ **C1**

Fig. 1: Chemical structures of the cited compounds.

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LIST OF PARTICIPANTS

Alcaro, Stefano	Università Magna Graecia di Catanzaro, Italy
Álvarez Pérez, Silvia	San Pablo CEU University, Spain
Álvarez-Builla, Julio	University of Alcalá, Spain
Anderluh, Marko	University of Ljubljana, Slovenia
Arany, Ádám	Semmelweis University, Hungary
Arsovska, Emilija	University of Ljubljana, Slovenia
Artese Anna	Università Magna Graecia di Catanzaro, Italy
Battisti, Umberto	University of Modena and Reggio Emilia, Italy
Bhattarai, Sanjay	University of Bonn, Germany
Boháč, Andrej	Comenius University Bratislava, Slovakia
Boshta, Nader	University of Bonn, Germany
Botta, Maurizio	University of Siena, Italy
Bottino, Paola	University of Catania, Italy
Brai, Anna Laura	University of Siena, Italy
Brasili, Livio	University of Modena and Reggio Emilia, Italy
Brus, Boris	University of Ljubljana, Slovenia
Brvar, Matjaž	University of Ljubljana, Slovenia
Buemi, Maria Rosa	University of Messina, Italy
Carrasco, Marta	University of Lisbon, Portugal
Chimirri, Alba	University of Messina, Italy
Ciancimino, Cristina	Università degli studi di Palermo, Italy
Cirrincione, Girolamo	Università degli studi di Palermo, Italy
Corrado, Sandra	University of Modena and Reggio Emilia, Italy
Cortes Cabrera, Alvaro	Universidad de Alcala, Spain
Costa, Elena	University of Padova, Italy
D'Aloja, Marco	University of Bari, Italy
De Clercq, Dries	University of Ghent, Belgium
De Micheli, Carlo	Università degli Studi di Milano, Italy
Defrenza, Ivana	University of Bari, Italy
Dela, Anna	Jagiellonian University Medical College, Poland
Demopoulos, Vassilis	Aristotle University of Thessaloniki, Greece
Diana, Patrizia	Università degli studi di Palermo, Italy
Dobricic, Vladimir	University of Belgrade, Serbia
Dolezal, Martin	Charles University in Prague, Czech Republic
Estevez Closas, Veronica	Universidad Complutense de Madrid, Spain
Fallacara, Anna Lucia	University of Siena, Italy
Franchini, Carlo	University of Bari, Italy
Fuchs, Alexander	University of Bonn, Germany
Gagni, Paola	Università degli Studi di Milano, Italy
Gazvoda, Martin	University of Ljubljana, Slovenia
Gobec, Stanislav	University of Ljubljana, Slovenia
Guillaume, Joren	University of Ghent, Belgium
Gütschow, Michael	University of Bonn, Germany
Guzior, Natalia	Jagiellonian University Medical College, Poland
Haider, Norbert	University of Vienna, Austria
Handzlik, Jadwiga	Jagiellonian University Medical College, Poland
Hodnik, Žiga	University of Ljubljana, Slovenia
Hrast, Martina	University of Ljubljana, Slovenia
Ilaš, Janez	University of Ljubljana, Slovenia

Introcaso, Antonellina	University of Bari, Italy
Jakopin, Žiga	University of Ljubljana, Slovenia
Ježko, Pavol	Comenius University Bratislava, Slovakia
Jukič, Marko	University of Ljubljana, Slovenia
Kamińska, Katarzyna	Jagiellonian University Medical College, Poland
Kikelj, Danijel	University of Ljubljana, Slovenia
Kolšek, Katra	University of Ljubljana, Slovenia
Korabecny, Jan	Charles University in Prague, Czech Republic
Kratochvíl, Jiří	Charles University in Prague, Czech Republic
Li, Wenjin	University of Bonn, Germany
Maccioni, Elias	University of Cagliari, Italy
Malerba, Paola	University of Bari, Italy
Manevski, Nenad	University of Helsinki, Finland
Marottoli, Roberta	University of Bari, Italy
Mastronardi, Federica	Università degli Studi di Milano, Italy
Mattei, Patrizio	F Hoffmann La Roche AG, Switzerland
Mátyus, Péter	Semmelweis University, Hungary
Meleddu, Rita	University of Cagliari, Italy
Merkač, Barbara	National Institute of Chemistry, Slovenia
Moraca, Federica	Università Magna Graecia di Catanzaro, Italy
Moreira, Rui	University of Lisbon, Portugal
Morreale, Francesca	University of Messina, Italy
Mravljak, Janez	University of Ljubljana, Slovenia
Müller, Christa E.	University of Bonn, Germany
Obreza, Aleš	University of Ljubljana, Slovenia
Oliveira, Rudi	University of Lisbon, Portugal
Otrębska, Ewa	Jagiellonian University Medical College, Poland
Ottersbach, Philipp Aaron	University of Bonn, Germany
Panagiota, Karmou	University of Athens, Greece
Parrino, Barbara	Università degli studi di Palermo, Italy
Pascual-Teresa Fernandez, Beatriz	San Pablo CEU University, Spain
Peterlin-Mašič, Lucija	University of Ljubljana, Slovenia
Rempel, Viktor	University of Bonn, Germany
Ribaudo, Giovanni	University of Padova, Italy
Ribelles Torres, Pascual	Universidad Complutense de Madrid, Spain
Ronsisvalle, Giuseppe	Università degli Studi di Catania, Italy
Rycek, Lukas	Vienna University of Technology, Austria
Salisova, Marta	Comenius University Bratislava, Slovakia
Sanchez Alonso, Patricia	Universidad de Alcala, Spain
Savić, Jelena	University of Belgrade, Serbia
Servusova, Barbora	Charles University in Prague, Czech Republic
Sollner-Dolenc, Marija	University of Ljubljana, Slovenia
Staderini, Matteo	Universidad Complutense de Madrid, Spain
Škedelj, Veronika	University of Ljubljana, Slovenia
Tomašić, Tihomir	University of Ljubljana, Slovenia
Trstenjak, Uroš	University of Ljubljana, Slovenia
Tsantili-Kakoulidou, Anna	University of Athens, Greece
Turnaturi, Rita	University of Catania, Italy
Vajs, Jure	University of Ljubljana, Slovenia

Vallianatou, Theodosia	University of Athens, Greece
Vinšova, Jarmila	Charles University in Prague, Czech Republic
Zega, Anamarija	University of Ljubljana, Slovenia
Zidar, Nace	University of Ljubljana, Slovenia
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Zega, A.	O-21
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