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Welcome

Dear participant

On behalf of the European Federation for Medicinal Chemistry (EFMC) and the Organising Committee, we warmly welcome you to Ljubljana for the 5th edition of the EFMC Young Medicinal Chemist Symposium (EFMC-YMCS).

Since the first edition of the EFMC-YMCS in 2014, the symposium never stopped expanding with more participants each time, and a programme enriched by an always increasing participation of EFMC-National Adhering Organisations.

However, the initial aim of this project remained:
• Creating a network of young European investigators in Medicinal Chemistry
• Stimulating young European investigators in Medicinal Chemistry to share their scientific work with peers and inspiring leaders in the field
• Creating competition and excellence in Medicinal Chemistry within Europe by selecting the European Champion in Medicinal Chemistry

This year, more than 170 scientists coming from 30 nations will gather in Ljubljana for yet another successful edition of this exciting mini-symposium. The 2 keynote lectures, 20 oral communications given by invited prize winners from national young medicinal chemist meetings in Europe, 2 additional oral communications selected from submitted abstracts, 20 Flash Poster Presentations and more than 100 poster presentations will lead you through the latest drug discovery advances in the major therapeutic areas.

The Organising committee is also happy to welcome registered participants to the newly created “Networking and Career Event” where you will have the opportunity to interact with your peers and build your network in a relaxed atmosphere.

During the closing Award ceremony, the following prizes will be awarded to the European Champions in Medicinal Chemistry:
• EFMC-YMCS Presentation Prize, sponsored by the EFMC & Idorsia
• EFMC-YMCS Poster Prizes, sponsored by MDPI
• EFMC-YMCS Public’s Prize, sponsored by Roche

We thank our sponsors (Acies Bio, Evotec, Heptares Therapeutics, Idorsia, Janssen, KRKA, Lek and Roche), the University of Ljubljana and all the participating national adhering organisations for their support, and we look forward to your active participation!

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EFMC President

Dr Tihomir Tomašič
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Programme

Thursday September 6, 2018

13:45 Registration
14:30 Welcome and opening  
Session Chair: Dr Tihomir TOMASIC (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)
14:35 KL01 - From Hits, to Leads to Chemical Probes: An Exciting Journey  
Prof. Cristina NEVADO (UNIVERSITY OF ZURICH, Zurich, Switzerland)

YMCS competition presentation session I
Session Chair: Dr Daniel MERK (ETH ZÜRICH, Zürich, Switzerland)
15:05 OC01 - Impact and Prediction of Binding Kinetics on in Vivo Drug Efficacy  
Winner of the young medicinal chemist meeting in France  
Dr Abdennour BRAKA (IRIM, Montpellier, France)
15:25 OC02 - Prospective Applications of Artificial Intelligence in de Novo Molecular Design for Drug Discovery  
Mr Lukas FRIEDRICH (ETH ZURICH, Zurich, Switzerland)
15:45 OC03 - Targeted Metabolomics Profiling as a Basis for Predictive Models in Multiple Sclerosis Research  
Winner of the young medicinal chemist meeting in Russia  
Mr Marat KASAKIN (INSTITUTE OF CHEMICAL BIOLOGY AND FUNDAMENTAL MEDICINE, Novosibirsk, Russia)
16:05 Flash poster presentations (0-10)
FP01 - Targeting Basic Defect in Cystic Fibrosis: Discovery and Development of Novel Nanomolar F508-Del CFTR Correctors  
Dr Alejandra RODRIGUEZ-GIMENO (ITALIAN INSTITUTE OF TECHNOLOGY, Genova, Italy)
FP02 - Buruli Ulcer and the mTOR Pathway: Total Synthesis, Structure-Activity and Target Elucidation Studies of Mycolactones  
Dr Matthias GEHRINGER (UNIVERSITY OF TUEBINGEN, Tuebingen, Germany)
FP03 - The Development of New Treatments for Multi-Drug Resistant Tuberculosis  
Ms Lisa BARBARO (MONASH UNIVERSITY, Reservoir, Australia)
FP04 - Synthesis and Characterisation of Psoralen Derivates as Inhibitors of The β5i Subunit of the Immunoproteasome  
Ms Eva Shannon SCHIFFRER (UNIVERSITY OF LJUBLJANA, Zirovnica, Slovenia)
FP05 - Fragment-Based Approach Applied to The Discovery of Protein-Protein Interaction Stabilisers  
Mr Dario VALENTI (TAROS CHEMICALS GMBH & CO. KG, Dortmund, Germany)
FP06 - The Use of Irreversible Ligands in the Quest to Obtain the First Ligand-Bound X-Ray Structures of The Adenosine A1 Receptor  
Dr Manuela JORG (MONASH UNIVERSITY, Parkville, Australia)
FP07 - Inhibitors of Human Sialytransferases as Novel Anti-Metastatic Agents  
Mr Chris DOBIE (UNIVERSITY OF WOLLONGONG, Gwynneville, Australia)
FP08 - 3-Oxabicyclo[4.1.0]Heptane: A Bioisostere for Morpholine as a Kinase Hinge Binding Moiety  
Mr Declan SUMMERS (GLAXOSMITHKLINE, Stevenage, United Kingdom)
FP09 - Development of Small-Molecule Inhibitors of Adipose Triglyceride Lipase (ATGL)  
Ms Anna MIGGLAUTSCH (GRAZ UNIVERSITY OF TECHNOLOGY, Graz, Austria)
FP10 - Covalent Fragment-Based Discovery of New Mura Inhibitors  
Ms Martina HRAST (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)
16:25 Coffee break
Programme

YMCS competition presentation session II
Session Chair: Dr Kristina GONCHARENKO (SPIROCHEM, Basel, Switzerland)

16:40 OC04 - The Design and Synthesis of Bromodomain Photoaffinity Probes
Winner of the young medicinal chemist meeting in the United Kingdom
Mr David FALLON (GLAXOSMITHKLINE, Stevenage, United Kingdom)

17:00 OC05 - Gemini-Type Protacs: a Small Molecule-Based Strategy for Self-directed Inactivation of Cereblon
Mr Christian STEINEBACH (UNIVERSITY OF BONN, Bonn, Germany)

17:20 OC06 - Investigation of Direct Effect of Psychoactive Compounds on Invertebrate Neurons in Real Time
Winner of the young medicinal chemist meeting in Hungary
Dr Gabor MAASZ (HUNGARIAN ACADEMY OF SCIENCES, Budapest, Hungary)

17:40 OC07 - Hydrogen Peroxide Sensitive Prodrugs of Methotrexate and Aminopterin for the Treatment of Rheumatoid Arthritis
Winner of the young medicinal chemist meeting in Denmark
Dr Jorge PEIRO CADAHIA (NUEVOLUTION, Copenhagen, Denmark)

18:00 Flash poster presentations (10-20)

FP11 - Rational Design of Optimized Allosteric Effectors of Cathepsins K and S Based on Succinimide-Glycinate Scaffold
Ms Tjasa GORICAN (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

FP12 - Covalent Inhibition with Terminal Alkyne As 'Inert' Electrophile
Mrs Elma MONS (LEIDEN UNIVERSITY MEDICAL CENTER, Leiden, The Netherlands)

FP13 - Exploration of Chemical Space of Two Classes of INHA Inhibitors
Dr Izidor SOSIC (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

FP14 - Late-Stage Functionalization of Peptides: Novel Site-Selective Modification & Bioconjugation
Dr Anais NOISIER (ASTRAZENECA, Malmö, Sweden)

FP15 - Identification of A GSK-3β/CK-1δ Inhibitor: Dual Target and Dual Mechanisms of Action as Possible Strategy for the Treatment of Parkinson's Disease
Dr Federico STEPHANIE (UNIVERSITY OF TRIESTE, Trieste, Italy)

FP16 - Discovery of Benzothiazole-Based DNA Gyrase and Topoisomerase IV Inhibitors with Broad Spectrum Antibacterial Activity
Ms Martina DURCIK (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

FP17 - Development of Peptides as Therapeutics for Pharmacological Intervention in Vital Protein Cascades
Dr Christina LAMERS (UNIVERSITY OF BASEL, Basel, Switzerland)

FP18 - Deep Learning Applications in The Design and Identification of Antibacterial Compounds
Dr Marko JUKIC (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

FP19 - The Design and Synthesis of Novel Inhibitors of the Notch Activation Complex Kinase for Notch Mediated Tumorigenesis
Ms Tanya KELLEY (UNIVERSITY OF MIAMI, Miami, United States)

18:20 Poster presentation session 1 & Networking drink
20:00 End of the day
20:15 Optional Networking Event (until 21:30)
Programme

Friday September 7, 2018

YMCS competition presentation session III
Session Chair: Dr Ziga JAKOPIN (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

08:30  OC08 - Fosmidomycin Analogs as Antimalarial and Antitubercular Agents - a Prodrug Approach  
Winner of the young medicinal chemist meeting in Belgium (KVCV)  
Ms Charlotte COURTENS (UGENT, Ghent, Belgium)

08:50  OC09 - Metal-Chelating Acetohydroxamic Acids against Hepatitis C Virus and Flaviviruses  
Winner of the young medicinal chemist meeting in Greece  
Ms Erofili GIANNAKOPOULOU (NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS, Athens, Greece)

09:10  OC10 - Elucidation of the Antifungal Mechanism of Action of Bis-Guanylhydrazones  
Winner of the young medicinal chemist meeting in Serbia  
Ms Jelena LAZIC (UNIVERSITY OF BELGRADE, Belgrade, Serbia)

09:30  OC11 - Discovery and Optimisation of N-Phenylpyrrolamides as DNA Gyrase B Inhibitors  
Winner of the young medicinal chemist meeting in Slovenia  
Dr Nace ZIDAR (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

09:50  OC12 - The Effect of Singlet Oxygen Production and Lipophylicity of the Photosensitizer in Photodynamic Activity of N-Methylated and N-Oxidised Pyridylporphyrins  
Winner of the young medicinal chemist meeting in Croatia  
Ms Martina MUSKOVIC (UNIVERSITY OF RIJEKA, Rijeka, Croatia)

10:10  Coffee Break

YMCS competition presentation session IV
Session Chair: Mr Roman SINK (UNIVERSITY OF LJUBLJANA, Ljubljana, Slovenia)

10:30  OC13 - Synthesis and Biological Evaluation of Bia 10-2474: an Irreversible and Aspecific Faah Inhibitor  
Winner of the young medicinal chemist meeting in The Netherlands  
Mr Anthe JANSSEN (LEIDEN UNIVERSITY, Leiden, The Netherlands)

10:50  OC14 - Methylene- Cycloalkylacetate (mca) as Novel Neurotropic Agents  
Winner of the young medicinal chemist meeting in Israel  
Mr David LANKRI (HEBREW UNIVERSITY, Jerusalem, Israel)

11:10  OC15 - Development of Release-and-Report Kinase Inhibitors as Molecular Tools for Investigating Neurodegenerative Disorders  
Winner of the young medicinal chemist meeting in Sweden  
Ms Cassandra LEE FLEMING (UNIVERSITY OF GOTHENBURG, Göteborg, Sweden)

11:30  OC16 - Piperazine Derivatives as a Novel, Active Histamine H3 Receptor Ligands  
Winner of the young medicinal chemist meeting in Poland  
Mrs Katarzyna SZCZEPAŃSKA (JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE, Krakow, Poland)

11:50  Lunch & Poster presentation session 2
Programme

YMCS competition presentation session V
Session Chair: Dr Emmanouil FOUSTERIS (UNIVERSITY OF PATRAS, Patras, Greece)

13:15 OC17 - Development of Aminoxynone as the First-in-Class Hsp90 C-Terminal Domain Dimerization Inhibitor
Winner of the young medicinal chemist meeting in Germany
Prof. Finn HANSEN (UNIVERSITY OF LEIPZIG, Leipzig, Germany)

13:35 OC18 - How Can We Inhibit a Protein that is Intrinsically Disordered? Androgen Receptor – EPI-001 a Case Study
Winner of the young medicinal chemist meeting in Spain
Mrs Marta FRIGOLE-VIVAS (INSTITUTE FOR RESEARCH IN BIOMEDICINE (IRB), Barcelona, Spain)

13:55 OC19 - Kinase Templated Abiotic Reaction: from Promiscuity to Selectivity
Winner of the young medicinal chemist meeting in Switzerland
Mr Jacques SAARBACH (UNIVERSITY OF GENEVA, Geneva, Switzerland)

14:15 OC20 - The Best of Three Worlds: Tackling Multidrug Resistance through Phytochemical, Biological and Computational Approaches
Winner of the young medicinal chemist meeting in Portugal
Dr Ricardo FERREIRA (UPPSALA UNIVERSITY, Uppsala, Sweden)

14:35 OC21 - Targeting Zinc-Finger Proteins in Cancer and Viral Infections
Winner of the young medicinal chemist meeting in Italy
Dr Mattia MORI (UNIVERSITY OF SIENA, Siena, Italy)

14:55 Voting break

15:10 KL02 - My Lessons in Drug Discovery
Dr Marton CSEKEI (SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, Budapest, Hungary)

15:40 Closing and award ceremony
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Keynote Lectures
FROM HITS, TO LEADS TO CHEMICAL PROBES: AN EXCITING JOURNEY

Cristina Nevado (1), Amedeo Caflisch (2), Aymeric Dolbois (1), Vlad Pascanu (1), Pawel Sledz (1), Laurent Batiste (1), Unzue Andrea (1)

1) Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH 8057 Zurich, Switzerland
2) Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH 8057 Zurich, Switzerland

Chemical modifications of histones, the proteins around which the genetic material (DNA) is wrapped, provide an important regulatory layer for gene expression control. In this context, bromodomain proteins function as readers of specific chemical modifications on the histone tails. Given the direct connection between the regulation of gene expression and physiological and pathological processes, molecules interfering with readout of these modifications by the bromodomains have recently emerged as important chemical probes and clinical tools to fight cancer, inflammation and other diseases.

Here, we will present our efforts towards a modular compound-generation and validation platform for rapid design, synthesis and characterization of potent and selective chemical inhibitors and their optimization and validation as chemical probes.
Márton Csékei joined Servier Research Institute in Budapest as a synthetic chemist right after finishing the preparative work of his PhD. During the years he had the opportunity to gain some experience in medicinal chemistry.

For an industrial chemist, it is very challenging to find topics, which can be shared outside the company. In the presentation he will show some lessons, challenges and their solution through his way of becoming a medicinal chemist.
Computational and Structural Biotechnology Journal (CSBJ) is an online open-access journal publishing research articles and reviews after full peer review (ISSN 2001-0370). Specific areas of interest include, but are not limited to:

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Oral Communications
IMPACT AND PREDICTION OF BINDING KINETICS ON IN VIVO DRUG EFFICACY

Abdennour Braka (1,2), Samia Acı-Sèche (1), Stéphane Bourg (1), Karen Plé (1), Anthony Champiré (1), Aurélie Cosson (2), Béatrice Vallée (2), Hélène Bénédetti (2), Sylvain Routier (1), Norbert Garnier (2), Pascal Bonnet (1)

1) Institut de Chimie Organique et Analytique (ICOA), Université d’Orléans, CNRS, UMR 7311, 45067 Orléans, France.
2) Centre de Biophysique Moléculaire, CNRS, UPR 4301, Rue Charles Sadron, 45071 Orléans Cedex 2, France.

Historically, some drug design programs have focused on the optimization of drug candidates based on structure-activity relationships (SARs) as an approximation of in vivo efficacy. However, the efficacy of a ligand is not always adequately described by the SAR because it mainly depends on the lifetime of in vivo interactions between the ligand and its receptor. Today, structure-kinetic relationships (SKR) are of major interest for the discovery of new drugs, particularly in the early stage of optimization of molecules in order to better evaluate their safety and efficacy.

With the growing interest of SKRs, we propose a new methodology to understand the mechanism of the binding of a ligand with its biological target in order to predict its kinetic constants.

Our method begins by identifying the binding pathways of the molecules. The simulation of the binding process is carried out using adaptive steered molecular dynamics (ASMD) to drive the ligand from/to the binding site. Next, we sample the conformational space along the simulated paths to get a relevant statistical distribution of system states. From this statistical distribution, the free energy profile is evaluated using the weighted histograms method (WHAM). Based on the transition state theory, the energy profile of the simulations will be used to predict the kinetic constants (kon and koff) and to identify the structural determinants responsible for the energetic barriers detected during the simulations.
Modern instances of artificial intelligence (AI) (e.g., deep learning) and the availability of large chemical and biological datasets enable the development of innovative concepts in drug discovery and development. We have applied a so-called “generative” deep learning model based on a deep recurrent neural network (RNN) containing long short-term memory (LSTM) for de novo molecular design. This computational model was first trained to capture the grammar of SMILES representations of bioactive small molecules, and then used to automatically generate SMILES strings of new chemical entities (NCEs). By means of transfer learning, the model could be fine-tuned to create target-focused sets of molecules. In a pioneering prospective study, the generative RNN was trained on bioactive molecules (540,000) from a public compound database (ChEMBL22) and further fine-tuned with a small set of 25 known agonists of retinoid X and peroxisome proliferator-activated receptors (RXR, PPAR). The de novo designs generated by this model were ranked computationally, and five top-ranked compounds were synthesized. Four out of five molecules showed nano- to micromolar potencies against the studied targets (RXR and/or PPAR) with distinct activity profiles. In further prospective studies, we applied generative AI models to the de novo design of bioactive natural product mimetics. The computer-generated NCEs resemble structure of the natural product and inherited the bioactivity profile of their template. Our results highlight generative AI as an innovative knowledge-driven approach to obtain pharmacologically relevant NCEs.

References
1) E. Gawehn, J. A. Hiss, G. Schneider, Mol. Inf. 2016, 35, 3–14
2) A. Gupta, A. T. Müller, B. J. H. Huisman, J. A. Fuchs, P. Schneider, G. Schneider, Mol. Inf. 2018, 37, 1700111
4) G. Schneider, Nat. Rev. Drug Discov. 2018, 17, 97-113
TARGETED METABOLOMICS PROFILING AS A BASIS FOR PREDICTIVE MODELS IN MULTIPLE SCLEROSIS RESEARCH

Marat Kasakin (1,2), Artem Rogachev (2,3), Vladimir Koval (1), Elena Predtechenskaya (2), Andrey Pokrovsky (2)

1) Institute of Chemical Biology and Fundamental Medicine of SB RAS, 8 Lavrentiev Avenue, Novosibirsk, 630090, Russia
2) Novosibirsk State University 1 Pirogova street, Novosibirsk, 630090, Russia
3) N. N. Vorozhtsov Novosibirsk institute of organic chemistry, 9 Lavrentiev Avenue, Novosibirsk, 630090, Russia

Multiple Sclerosis (MS) is one of the autoimmune disorders causing demyelination of axons and impacting on the central nervous system. Modern diagnostics of MS is based on clinical decision according to revised MacDonald criterion including MRI method to confirm the result [1]. In spite of many risk factors of MS is known, the progress of the disease after the first clinical symptoms is difficult to predict, therefore the development of new methods for diagnosis and status monitoring is an actual topic. Amino acids and fatty acids metabolism are disturbed during MS [2], which makes biomarkers search among the relative metabolites to be prospective for applying in diagnosis. Metabolomics and multivariate statistical analysis is a powerful approach for discovering biomarkers and investigation of human disease mechanism[3].

We performed targeted metabolomics approach based on quantitative LC-MS/MS analysis of amino acids and acylcarnitines in dried plasma samples followed by multivariate statistical analysis using R integrated suite for discovering differences between MS (n=16) and control (n=12) groups. It was found that partition least square discriminant analysis (PLS-DA) method gives better separation between the groups comparing to principal component linear discriminant analysis (PCA-LDA) algorithm (Figure 1), although it could be overestimated during leave-one-out cross-validation and needs to evaluation on the test group. Predictive models yield to AUC = 0.79, Sensitivity = 0.67, Specificity = 0.75 for PCA-LDA; 0.98, 0.81, 1 for PLS-DA and 0.80, 0.64, 0.80 for random forest algorithm (RF), respectively. PLS-DA model performs preliminarly the best results for MS identification, PCA-LDA and RF models produce results close to each other. All three models detect noticeable changes in amino acids and acylcarnitines profile in MS group in comparison with control group.

Metabolomics approach and several multivariate statistical algorithms were shown to be successful in solving the classification problem, particularly in separation of MS and control group samples based on differences in amino acids and acylcarnitines profile. The results obtained are promising for further development of the clinical decision support system.

References
THE DESIGN AND SYNTHESIS OF BROMODOMAIN PHOTOAFFINITY PROBES

David Fallon (1,2), Jacob Bush (1), Nicholas Tomkinson (2)

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2) University of Strathclyde, Glasgow, UK

In recent years, photoaffinity probes have become powerful tools in the field of chemical biology, allowing a greater understanding of the proteome. These tools can provide a wealth of information regarding on/off target affinity, especially when coupled with modern MS-based proteomics. Inherently, photoaffinity probes are highly functionalised molecules, usually containing an affinity function, photoreactive moiety, and a bio-orthogonal handle. The stability, compatibility and ease-of-synthesis of these tri-functional molecules can prove extremely challenging. A general protocol for the bringing-together of the three separate functional components in a single synthetic step to synthesise fully elaborated photoaffinity probes has not been reported.

Herein we present the use of the Ugi reaction to combine various commercially available photoreactive groups with a Bromodomain-targeting chemical probe in a single synthetic step. This robust protocol allowed for a two-dimensional synthetic array to study the photocrosslinking yields of five commonly used photoreactive groups, in combination with three different linker-lengths. The results and implications from this linker length study are discussed, along with the direct applications of these bromodomain-targeting photoaffinity probes. This includes their use in high-throughput assays with recombinant protein, and live cell target engagement studies with a MS-based proteomic readout.
GEMINI-TYPE PROTACS: A SMALL MOLECULE-BASED STRATEGY FOR SELF-DIRECTED INACTIVATION OF CEREBLON

Christian Steinebach (1), Stefanie Lindner (2), Jan Krönke (2), Michael Gütschow (1)

1) Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany
2) Department of Internal Medicine III, University Hospital Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany

The ability of a small-molecule to target a protein for degradation is an exciting implication for modern drug discovery. One such approach is based on the chemical-induced degradation of disease-causing proteins by heterobifunctional molecules, referred to as Proteolysis Targeting Chimeras (PROTACs) which induce ubiquitination and targeted proteasomal degradation.1 We sought for a chemical knockdown of the E3 ligase cereblon (CRBN), the target of thalidomide and its analogs lenalidomide and pomalidomide. Thus, we developed gemini-type PROTACs that were designed for a self-directed inactivation of CRBN via ternary complex formation, subsequent ubiquitination and degradation. For this purpose, two identical CRBN binding moieties, i.e. pomalidomide, were connected with different linkers via the amino group of each ligand. The chemical 1+2 assembly of the final compounds was accomplished by a nucleophilic substitution of two 4-fluoro thalidomides with several α,ω-diamino linker building blocks.

The resulting gemini-type PROTACs were studied with respect to the expected induction of a CRBN ubiquitination and degradation. Western blot analyses revealed a reduced CRBN content of treated cells depending on the chemical structure of the applied homodimers. Moreover, known neosubstrates of CRBN, i.e. transcription factors IKZF1 and IKZF3,2,3 were also investigated to assess the suitability of our probes. Some of our compounds were characterized as efficient CRBN degraders with only minimal effects on IKZF1 and IKZF3 depletion. Based on these properties, a selected probe was subjected to a global proteomic analysis. Such gemini-type PROTACs might be a considerable leap forward to unravel the biological complexities of CRBN and to provide additional insights into the mechanism of CRBN-targeting drugs.

References
2) Krönke et al., Science, 2014, 343, 301
3) Krönke et al., Nature, 2015, 523, 183
INVESTIGATION OF DIRECT EFFECT OF PSYCHOACTIVE COMPOUNDS ON INVERTEBRATE NEURONS IN REAL TIME

Gabor Maasz

NAP Adaptive Neuroethology, Department of Experimental Zoology, Balaton Limnological Institute, MTA-Centre for Ecological Research, 8237 Tihany, Hungary

High throughput and in situ analysis of drug’s effect is an analytical challenge. It is hard to perform investigation, which is in situ and real time together. Generally, methodology implementation is complicate or partly suitable.

Central nervous system (CNS) of the great pond snail (Lymnaea stagnalis), which species is an invertebrate OECD (Organisation for Economic Co-operation and Development) model animal for testing chemicals, was used for the investigation. For the analysis, HPLC-EP-MS real time method (1) and metabolomic analysis of single-cell by capillary microsampling combined mass spectrometry system (2) were used.

1. Separation of complex samples into their single components by HPLC and subsequent fraction collection paired with electrophysiological (EP) testing on live cells is an already established method. However, the development of HPLC-EP-MS online coupled system for investigation of pharmacokinetic characteristics allows us to acquire quantitative information about analytes and their physiological effects on identified neurons in real time. Our goal was to create an online system which can combine the advantages of electrophysiology and mass spectrometry. Further aim was to test the utility of novel system on the central nervous system of L. stagnalis.

The system consists of an Infinity 1260 LC system (Agilent Technology), an AmaZon SL ion trap (Bruker Daltonics GmbH) and an electrophysiology rig (AxoClamp 900A amplifier, DigiData 1550 digitizer; Axon Instruments). An inert and live dye (Fast Green) was used for monitor the direction of the flow under a light microscope. The system was tested with acetylcholine and we were able to reproduce a known neuronal effect of Chelidonium majus. Active substances were quantitatively determined.

This method allows us to simultaneously acquire quantitative information of the analytes and their effects on the CNS. The system is able to measure the neurotoxicological sensitivity as well as neuropharmacological and pharmacokinetic parameters. The dynamics of neurochemical processes could also be tested at the system level in L. stagnalis CNS.

2. Capillary microsampling is a direct technique that utilizes a capillary tip to extract cell content to be analyzed by MS, and also enables probing the metabolites, lipids and peptides located in specific regions of a cell. This combined method allows us to analyze single cells by MS, and the feasibility of this approach has been demonstrated in experiments aiming to determine the biomolecular composition of cultured single cells in different organisms, such as mammalian and plant cells. Due to its high sensitivity and specificity, capillary microsampling combined MS method is an excellent tool for non-targeted biomolecular analysis of single cells. This method was already successfully used on L. stagnalis neurons and with this the direct effect of psychoactive agent is also able to be investigated.

Acknowledgement

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HYDROGEN PEROXIDE SENSITIVE PRODRUGS OF METHOTREXATE AND AMINOPTERIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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A series of novel hydrogen peroxide sensitive prodrugs of methotrexate (MTX) and aminopterin (AMT) were synthesized and evaluated for therapeutic efficacy in mice with collagen induced arthritis (CIA) as a model of chronic rheumatoid arthritis (RA). The prodrug strategy selected is based on reactive oxygen species (ROS)-labile 4-methylphenylboronic acid promoieties linked to the drugs via a carbamate linkage or a direct C–N bond. Activation under pathophysiological concentrations of H2O2 proved to be effective, and prodrug candidates were selected in agreement with relevant in vitro physicochemical and pharmacokinetic assays. Selected candidates showed moderate to good solubility, high chemical and enzymatic stability, and therapeutic efficacy comparable to the parent drugs in the CIA model (Figure 1). Importantly, the prodrugs displayed the expected safer toxicity profile and increased therapeutic window compared to MTX and AMT while maintaining a comparable therapeutic efficacy, which is highly encouraging for future use in RA patients.

Figure 1. Methotrexate (MTX) and aminopterin (AMT) prodrugs and their efficacy in the collagen induced arthritis (CIA) model.

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FOSMIDOMYCIN ANALOGS AS ANTIMALARIAL AND ANTITUBERCULAR AGENTS - A PRODRUG APPROACH

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Antimalarial and antitubercular agents with new mechanisms of action are necessary to tackle infections by Plasmodium parasites and Mycobacteria that are resistant to current therapies. Fosmidomycin has been shown to be a well-tolerated, safe and efficacious antimalarial drug in combination treatment\(^1\). However, its pharmacokinetic (PK) properties are less than ideal, with only moderate bioavailability and a short plasma half-life\(^2\). Moreover, because of the unique highly lipophilic cell wall of Mycobacteria, fosmidomycin cannot cross the cell wall and thus, is not active against Mycobacteria\(^3\).

A lot of research has been dedicated to improve the potency of fosmidomycin analogs\(^4\). However, the problem of low bioavailability remains. The development of hydrophobic phosphonate and/or hydroxamate prodrugs of fosmidomycin could improve both oral bioavailability and cell penetration by passive diffusion. To date, only acyloxymethyl- and alkoxy-carbonyloxymethyl phosphonate prodrugs have been reported, both with only moderate in vivo activity\(^5,6\). The aim of this research is to design and synthesize a broad range of potential prodrugs with different bioactivation mechanisms in order to enhance in vivo antimalarial and antitubercular activity as a result of optimized PK properties.

This research demonstrates that a prodrug approach may allow to convert fosmidomycin into agents with improved permeability characteristics, opening avenues for its use as antimalarial and/or antitubercular drug. Further optimization of the prodrug pro-moiety is however still needed to obtain more potent analogs, especially with regard to whole cell antitubercular activity.

References

METAL-CHELATING ACETOHYDROXAMIC ACIDS AGAINST
HEPATITIS C VIRUS AND FLAVIVIRUSES

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Hepatitis C Virus (HCV) infections pose a major public health threat globally, with infected individuals being at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. There is no vaccine available and despite advances in current chemotherapy, the global burden of HCV infections remains high, due to their partial effectiveness or viral resistance. The flaviviruses Dengue (DENV), Yellow fever (YFV), and the re-emerging Zika virus (ZIKV) cause diseases ranging from mild febrile illness to severe encephalitis or hemorrhagic syndromes. Despite the extensive research on flaviviral diseases, there is no clinically approved therapy, thus, they constitute high priority targets for drug discovery. Because of all the above and based on literature reports on metal-chelating agents inhibiting HCV NS5B-polymerase,[1],[2] the development of novel scaffolds of metal-chelators with antiviral properties was undertaken.

By utilizing docking-scoring calculations, structural insight regarding HCV inhibition was obtained, prompting the rational design and synthesis of novel carbocyclic-(spiro)substituted hydantoin-derivatives, bearing the acetohydroxamic acid metal-chelating group upon the imidic nitrogen, and a variety of lipophilic substitutions at the amidic nitrogen atom.

The compounds were evaluated for their effect on HCV RNA replication and cell viability (ATP and luciferase assays), exhibiting EC50 values ranging from 0.08 to 4.50 μM, in Huh7 reporter subgenomic replicon cell lines of genotype 1b, and remarkable Selectivity Indexes rising up to 781. The fact that flaviviruses are members of the Flaviviridae family, along with HCV, and they share several similarities among their homologous metalloenzymes (NS5B/NS5 RNA-dependent RNA polymerase and NS3 protease/helicase)[3],[4] prompted the evaluation of the most potent anti-HCV compounds against DENV, YFV and ZIKV.

The preliminary anti-flaviviral results of low μM EC50 values, observed for many compounds (representative EC50 values 0.07 μM, 2.76 μM, and 0.44 μM for DENV, YFV and ZIKV respectively), are highly encouraging and, along with theoretical simulations, suggest that the novel framework of metal-chelators we developed offers a highly promising starting point for the design of potent and broadly effective antiviral agents with dual-target potential. Analysis of resistance mutations and modeling studies are currently underway to further characterize their inhibition mechanism.

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ELUCIDATION OF THE ANTIFUNGAL MECHANISM OF ACTION OF BIS-GUANYLHYDRAZONES

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The incidence of fungal infections (Candida spp. and Aspergillus spp.) is increasing exponentially and their outcome is lethal in over one million cases per year.¹ The drug that is mainly used for combating these infections is Amphotericin B, but its nephrotoxic side effects are a hindering factor.² Therefore, it is imperative to look into the possibility of combinational therapy and discover novel antifungals with an alternative mechanism of action to the one of Amphotericin B.

Following the previous discoveries of our research group showing a fungistatic effect of one novel bis-guanylhydrazone that affected C. albicans biofilm formation and disruption, together with the good cytotoxicity and embryo toxicity profile³, we synthesized three novel compounds, each one containing two guanylhydrazone functional groups.

The antifungal screening (C. albicans, C. glabrata, C. parapsilosis) of the novel compounds showed great minimal inhibitory concentration (MIC) values against planktonic cells, and inhibitory effects against the formation of C. albicans biofilms. Further on, we showed interaction with the fungal DNA using in vitro tests, circular dichroism experiments and molecular modeling. The most promising compound was shown to be a DAPI (small groove binding stain) competitor, and after the initial interaction with the fungal DNA, death of C. albicans cells was caused by the activation of metacaspases leading to apoptosis. In addition, this compound showed a synergistic effect with Amphotericin B, which makes it a promising antifungal and opens possibilities for further research.

Acknowledgements

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References

DISCOVERY AND OPTIMISATION OF N-PHENYLPYRROLAMIDES AS DNA GYRASE B INHIBITORS

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In recent years, the increasing number of life-threatening infections due to resistant Gram-positive and Gram-negative pathogens is becoming more and more problematic. The ATP binding site located on the subunit B of DNA gyrase is an attractive target for the development of new antibacterial agents. Several small-molecule inhibitor classes have been discovered but none has so far reached the market.

Based on the structures of marine alkaloid oroidin, we have recently designed and prepared a series of N-phenyl-4,5-dibromopyrrolamides and N-phenylindolamides as ATP competitive DNA gyrase B inhibitors.1 Compounds were evaluated against DNA gyrase and topoisomerase IV from Escherichia coli and Staphylococcus aureus. A high-resolution crystal structure of one of the inhibitors in complex with E. coli DNA gyrase B (Figure 1a) revealed details of its binding mode within the active site. Compounds were evaluated against a diverse panel of Gram-positive and Gram-negative bacterial strains including some clinically important resistant strains. With structure-based design, we have prepared several optimized series of N-phenylpyrrolamides with improved on-target and antimicrobial activities.2-4 From the initial hits with micromolar IC₅₀ values against DNA gyrase, in several optimization cycles, we have prepared compounds with DNA gyrase inhibitory activities in the low nanomolar range. Minimum inhibitory concentrations (MICs) against selected Gram-positive and Gram-negative bacteria were in the low- or sub-micromolar range. The oxadiazolone 11a (Figure 1b), with an IC₅₀ value of 85 nM against E. coli DNA gyrase displayed MIC values of 1.56 μM against Enterococcus faecalis, and 3.13 μM against wild type S. aureus, methicillin-resistant S. aureus (MRSA) and vancomycin-resistant Enterococcus (VRE).4 Compounds were found to be selective against human topoisomerase II enzyme and to have low cytotoxicity.

**Figure 1.** a) X-ray crystal structure of E. coli DNA gyrase B in complex with N-phenylpyrrolamides inhibitor (PDB code: 4ZVI). The ligand and the protein are colored according to the chemical atom type (C in cyan, C in grey, N in blue, O in red, and Br in brown). The water molecule is presented as a red sphere.; b) Structure-based optimization of compound 11a and its inhibitory activity on DNA gyrase and on selected bacterial strains.

**References**

Photodynamic therapy (PDT) is based on cytotoxic action of singlet oxygen (\(^{1}\text{O}_2\)) and other reactive oxygen species (ROS), produced upon the visible light-excitation of a photosensitiser (PS), against cancer cells and pathogenic microorganisms.[1,2] Intracellular localisation of the PS is important for PDT efficiency, due to short lifetime (µs) and “working” distance (nm) of \(^{1}\text{O}_2\), and molecules that can easily penetrate into the cell are potentially better PSs.[3]

We have recently synthesised a series of \(N\)-methylated and \(N\)-oxidised tetra- and tripyridylporphyrins bearing 3- and 4-pyridyl groups, and we studied the effect of a long alkyl chain on their PDT activity. Photophysical characteristics proved to be very similar for all the compounds, as well as their ability to produce \(^{1}\text{O}_2\) using 9,10-dimethylantracen (DMA) photodegradation study.[4] A further study of the decrease of the absorption and fluorescence of 1,3-diphenylisobenzofuran (DPBF), in the presence of the photo-activated PSs, as a measure of \(^{1}\text{O}_2\) production will be described and compared to the relative method with DMA.

In contrast to their \(^{1}\text{O}_2\) production, the presence of a long alkyl chain and amphiphilicity of the PSs proved to be crucial for high PDT activity against five cancer cell lines. Furthermore, \(N\)-methylated tripyridylporphyrins were generally more PDT efficient than their N-oxidised analogues, and those with 3-pyridyl groups (TMPyP3-C\(_{17}\)H\(_{35}\) and TMPyP3-C\(_{17}\)H\(_{33}\)) were shown to be better PSs than those with 4-pyridyl groups.[4]

Cationic PSs can target Gram-negative bacteria due to electrostatic interactions,[5] however, the possible effect of a lipophilic chain is somewhat less investigated. Therefore, we compared the activity of amphiphilic TMPyP3-C\(_{17}\)H\(_{35}\) against Legionella pneumophila with the activities of two hydrophilic PSs, TMPyP3 and TMPyP3-CH\(_3\). The minimum inhibitory concentration (MIC) and minimum effective (MEC) values were determined in broth, and MEC in tap water, by the microdilution method. Violet and red light with different fluence rates and total doses were used for the activation of the PSs. The most efficient compound in all studies was amphiphilic PS while the activities of the hydrophilic PSs were significantly lower, though tetracationic TMPyP3 was slightly more efficient than tricationic TMPyP3-CH\(_3\). The complete inactivation of Legionella in sterile tap water was achieved with nanomolar concentration (0.024 \(\mu\text{M}\)) of TMPyP3-C\(_{17}\)H\(_{35}\), and the photosensitiser uptake assay has shown that this PS binds to the bacterial cell, already after 10 minutes of incubation in the dark.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF BIA 10-2474: AN IRREVERSIBLE AND ASPECIFIC FAAH INHIBITOR

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A recent phase 1 trial of the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474 led to the death of one volunteer and produced mild-to-severe neurological symptoms in four others. Although the cause of the clinical neurotoxicity is unknown, it has been postulated, given the clinical safety profile of other tested FAAH inhibitors, that off-target activities of BIA 10-2474 may have played a role. Here we use activity-based proteomic methods to determine the protein interaction landscape of BIA 10-2474 in human cells and tissues. This analysis revealed that the drug inhibits several lipases that are not targeted by PF04457845, a highly selective and clinically tested FAAH inhibitor. BIA 10-2474, but not PF04457845, produced substantial alterations in lipid networks in human cortical neurons, suggesting that promiscuous lipase inhibitors have the potential to cause metabolic dysregulation in the nervous system.1

References
METHYLENE - CYCLOALKYLACETATE (MCA) AS NOVEL NEUROTROPIC AGENTS

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Tricyclic spiroether structures can frequently be observed as scaffold segments of various biochemical compounds and drugs of natural origins. Examples of these structures have been identified among carbohydrates, terpenoids and alkaloids. Unfortunately, access to a large number of these target molecules and their structural analogues is either unknown or hindered by their multistep syntheses.

We realized that most of the tricyclic spiranoid ethers might be derived from a simple and common collective precursor via a controlled intramolecular sequence of transformations. We discovered that monocyclic diene-alcohol precursor (see scheme) could serve as such building block for their synthesis via controlled Pd-catalyzed cascade cyclization reactions. We demonstrated, for the first time, a simple link between diene-alcohol cores and diverse medium-sized spiroether architectures.

We have also noticed that precursors, employed as a platform for syntheses of spiroethers, possess the capacity to act as standalone cores of numerous natural products (such as dysidolide, halmic acid, angolensate and others). Our study, therefore, was inspired by the assumption that synthetic diene-alcohol scaffolds, which are small, rigid, and highly reminiscent of natural scaffolds, could serve as operational ligands for development of a neurotropic lead compound. Many diene-alcohol-based natural products have been firmly established to demonstrate pharmacological activities. Thus, we were motivated to apply our designed architectures to the discovery of novel neurotropic compounds using the pheochromocytoma (PC12) cell neuronal model. We investigated the neurotropic effect of a broad library of diene-alcohol and other related derivatives by comparison to NGF, a known neurotropic factor. Micrographs of the cells were collected by using a light microscope camera, and digitized photographs were analyzed for compound-induced neurotropic activity using an NIH image protocol. The results indicate that the alkene element, integrated within the cycloalkylacetate core, is indispensable for neurotropic activity. By employing this line of research, our ultimate aim is to single out a small molecules, bearing potential for treatment of brain disorders, caused by insufficient trophic support.

References
The aberrant regulation of lymphocyte-specific protein tyrosine kinases (LCK) has been associated with the over-activation of microglia cells (important immune effector cells that reside in the central nervous system, CNS) and in turn, the development of Alzheimer’s disease (AD). Unfortunately, the detail of LCK’s dynamic function and the importance of quantitative, spatial and time-dependent parameters regarding microglia activation are poorly understood. As such, the ability to manipulate LCK activity using light would result in temporal control of enzymatic activity, thus serving as a valuable approach to probe the function of LCK in microglia cells and in turn, further our understanding of AD and related neurodegenerative disorders.

While such studies cannot be performed using conventional LCK inhibitors, we are currently pursuing the development of a stimuli-responsive release-and-report system. This is to be achieved through the introduction of a photolabile caging moiety onto a fluorescent LCK inhibitor that exhibits ‘OFF-ON’ fluorescent changes in concert with the release and subsequent binding of the bioactive to the kinase enzyme (Figure 1). To date, a potent and selective LCK inhibitor that exhibits favourable fluorescent properties for cellular imaging has been developed. The inclusion of an appropriate caging group and its effects on kinase activity is the current focus of the project. In contrast to conventional LCK inhibitors, the development of the phoreponsive kinase inhibitor will allow us to gain spatiotemporal control over LCK activity as well as visualise the decaging process and subcellular localisation of the active kinase inhibitor.

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PIPERAZINE DERIVATIVES AS A NOVEL, ACTIVE HISTAMINE H3 RECEPTOR LIGANDS

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Histamine H3 receptors (H3R) are constitutively active G-protein coupled receptors mostly expressed in CNS. Interaction with these receptors results in modulation of histamine levels as well as that of other neurotransmitters. Therefore, blockade of these receptors might provide useful pharmacological target in treatment of many CNS-based diseases such as schizophrenia, Alzheimer and Parkinson’s disease, obesity, narcolepsy and attention-deficit hyperactivity disorder (ADHD) [1], also as dual acting ligands. [2]

During many years of research on active histamine H3 receptors ligands in Department of Technology and Biotechnology of Drugs (DTBD), the pharmacophore model for such compounds was developed. Basic core - mostly (homo)(methyl)piperidine moiety - is connected via an alkyl chain of variable length with the lipophilic part (an aromatic moiety). Figure 1.

Based on the results of the research so far, it is assumed that the 4-pyridylpiperazine moiety in the basic part of the compound determines their high affinity and selectivity for human H3R. [3] Moreover, the most promising compounds exhibited anticonvulsant activity in the maximal electroshock-induced seizure (MES) model in mice. Furthermore, pharmacological test results of selected ligand clearly indicate that it may affect the amount of calories consumed, thus act as an anorectic compound. Likewise its protective action against hyperglycemia and the development of overweight has been shown.

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References
DEVELOPMENT OF AMINOXYRONE AS THE FIRST-IN-CLASS HSP90 C-TERMINAL DOMAIN DIMERIZATION INHIBITOR

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Hsp90 has been discovered as a promising target to combat cancer and several inhibitors targeting the ATP binding pocket in the N-terminal domain (NTD) of Hsp90 are currently under clinical investigation.[1] Unfortunately, all N-terminal Hsp90 inhibitors trigger a resistance mechanism in cancer cells referred to as the heat shock response (HSR), which represents a major efficacy problem in clinical use. In contrast to Hsp90 NTD inhibitors, compounds that act at the C-terminal domain (CTD) of Hsp90 do not initiate the unfavorable HSR.[1] One novel approach to inhibit the CTD of Hsp90 is to target the Hsp90 CTD dimerization interface.[2,3] However, only a few peptidic CTD dimerization inhibitors have been described so far.[3]

We now utilized the knowledge about the folding propensity of α-aminoxy peptides[4] and recently discovered hot spots at the Hsp90 CTD dimerization interface[2] to design and synthesize the first peptidomimetic Hsp90 CTD dimerization inhibitors. The hit compound aminoxyrone (AX), an α-aminoxy hexapeptide, exhibited promising anti-proliferative and cytotoxic activity in several human myeloid leukemic cell line models including imatinib resistant cell lines.[5] The specificity of AX to Hsp90 was determined by a Hsp90-dependent luciferase refolding assay and further confirmed by analysis of the downstream signalling. Furthermore, binding of AX to the CTD of Hsp90 was demonstrated by MST measurements with the purified recombinant CTD of Hsp90. Most notably, in vivo proof of concept studies demonstrated the efficacy of AX at 0.5 mg/kg in a K-562-Luciferase Xenograft tumor model. AX reduced the tumor burden significantly with respect to tumor weight (AX: 0.2 ± 0.01g vs vehicle: 1.26 ± 0.44g (p = 0.04)). Immunoblot analysis of tumor samples derived from mice treated with AX revealed the absence of HSR. Taken together, we have developed AX as the first-in-class peptidomimetic Hsp90 CTD dimerization inhibitors.[5]

References
HOW CAN WE INHIBIT A PROTEIN THAT IS INTRINSICALLY DISORDERED? ANDROGEN RECEPTOR – EPI-001 A CASE STUDY

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Intrinsically disordered proteins (IDPs) are highly attractive drug targets1. However, targeting them is a major challenge as their lack of defined secondary and tertiary structures hinders conventional structure-based drug discovery.

Androgen Receptor (AR) is a hormone-activated transcription factor. AR N-terminal domain (AR-NTD) is intrinsically disordered. Its function is to recruit the basal transcription machinery to express genes related to the development of the male phenotype. AR over-activation leads to prostate cancer (PC) and, eventually, castration-resistant prostate cancer (CRPC) for which there is currently no treatment2.

EPI-001 is the only small molecule inhibitor of the AR-NTD and was identified by phenotypic screening3. A derivative of EPI-001 entered clinical trials for CRPC treatment. However, not much is known about its mechanism of action. In this project we want to understand how EPI-001 can specifically interact with the disordered AR-NTD. Also we are rationally building improved analogues and designing a screening assay to find new small-molecule scaffolds with the same mechanism.

So far, by NMR spectroscopy we have showed that EPI-001 interacts with a region of the NTD called Transactivation Unit-5, although with very low affinity4. In the symposium I will provide evidence that EPI-001 interacts with a specific conformational state of this domain that can be stabilized in vitro and closely resembles the state that this domain adopts in its biological milieu. Our results help understand the mode of action of this experimental drug and suggest general avenues for targeting proteins rich in intrinsic disorder such as transcription factors.

References
Protein kinases are essential regulators of cellular signalling and have been at the centre stage of drug discovery for the past decade. The successful development of kinase inhibitors against Abl demonstrated that kinases were drugable and triggered tremendous research effort in this area towards the whole kinome. However, inhibitors developed so far often target the conserved ATP binding site of the protein and thus are lacking selectivity, and the more selective ones are targeting an inactive form of the protein. These features limit their use as chemical probes to sense kinase activity.

Herein we report a strategy based on two reacting probes targeting both nucleotide and substrate binding sites. The reaction used allows to use fluorescence readout to selectively sense Abl of Src kinase activity both in biochemical assays and fixed whole cell experiments.

References
THE BEST OF THREE WORLDS: TACKLING MULTIDRUG RESISTANCE THROUGH PHYTOCHEMICAL, BIOLOGICAL AND COMPUTATIONAL APPROACHES

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Multidrug resistance (MDR) in cancer is one of the major impairments in the success of chemotherapy regimens. Accordingly, one of the most promising approaches in overcoming MDR is the development of efflux modulators for efflux pumps (1). However, a greater knowledge on the P-gp efflux mechanism is important to identify the key steps by which drug efflux occurs and how it can be prevented. The main objective of this work was to identify and optimize novel MDR reversers, derived from Euphorbia species, and to gain insights on the drug efflux mechanism by P-gp (2).

The phytochemical study of Euphorbia pedroi yielded four new diterpenes, two macrocyclic lathyranes, one macrocyclic jatrophan and a rearranged phorbol, together with a new spiropertene, several known terpenoids and two flavonoids. Moreover, two libraries of ent-abietane and flavanone derivatives were built through molecular derivatization of compounds isolated from E. pedroi. Chemical structures of compounds were deduced from physical and spectroscopic data (IR, MS and NMR experiments) (2).

The MDR reversal activity of compounds was evaluated by combining transport and chemosensitivity assays using the MDR1-transfected mouse T-lymphoma and Colo320 cell models. While several isolated compounds showed good MDR reversal activities, ent-abietane and flavanone derivatives also revealed increased potencies towards the MDR cell lines when compared with its parent compounds. The effects of flavanone derivatives on other ABC transporters was also assessed, with several compounds being selective for breast cancer resistance protein (BCRP) and multidrug resistance protein 1 (MRP1), respectively, and obtaining important structure-activity relationships toward more effective efflux modulators in both efflux pumps (2,3).

The efflux mechanism was also studied by means of molecular dynamics and docking studies. Based on a previously refined P-gp structure, three distinct drug-binding sites could be identified and characterized, in a good agreement with published experimental data. Drug transit from bulk water into the DBP was characterized as an overall free-energy downhill process, with no activation energy required for crossing the ‘entrance’ gate found between transmembrane domains. Furthermore, as substrates and modulators were show to have different free energies of adsorption in both lipid/water and protein/ water interfaces, important differences in drug-protein interactions, protein dynamics and membrane biophysical characteristics were able to be characterized (4).

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References
Zinc finger proteins are among the most abundant proteins in eukaryotes. Their functions are extraordinarily diverse and include DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly. (1) Although they share the common structural motif that is characterized by the coordination of one or more zinc ions, zinc finger structures are as diverse as their functions. During my postdoctoral research, I spent many efforts in the design and optimization of small molecules able to target specific zinc-finger proteins that are implicated in cancer and HIV/AIDS, respectively.

The zinc-finger glioma-associated oncogene-1 (Gli1) is the final and most powerful effector of the Hedgehog signaling pathway, whose aberrant expression has been linked to the onset and progression of several types of cancers. (2) By using a multidisciplinary approach that integrates computational modeling with natural products chemistry and molecular and cell biology, we discovered the natural compound Glabrescione B (GlaB) as the first direct inhibitor of Gli1 with anticancer activity in vitro and in vivo. (3)

The HIV-1 nucleocapsid protein (NC) is a highly conserved zinc-finger that is relevant for HIV-1 replication at multiple stages. Pharmacological inhibition of NC should provide strong antiretroviral effects and enhance the genetic barrier for drug resistance. (4) In the last ten years, several strategies to discover and optimize non-covalent NC inhibitors (NCIs) were implemented, including massive screening, rational design, and hit-to-lead optimization, also taking advantage of the European Community support (FP7). (5,6) A number of different scaffolds of NCIs were then investigated through classical medicinal chemistry cycles of design-synthesis-bioassay testing. Two of these chemotypes were further profiled in an early preclinical trial.

Overall, we contributed to move forward the knowledge on these particular zinc-finger proteins and to highlight their relevance in the development of novel anticancer or antiretroviral strategies, respectively. Research is still ongoing and new good results are expected to be delivered soon.

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Flash Poster Presentations
TARGETING BASIC DEFECT IN CYSTIC FIBROSIS: DISCOVERY AND DEVELOPMENT OF NOVEL NANOMOLAR F508-del CFTR CORRECTORS

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Cystic Fibrosis (CF) is a lethal genetic disease caused by mutations in the CF Transmembrane conductance Regulator (CFTR) chloride channel, resulting in reduced anion conductance on epithelial cells of multiple organs. Nearly 2000 mutations of the CFTR gene have been identified [1]; the most frequent is the deletion of phenylalanine at position 508 (F508-del). This mutation causes a severe defect in protein folding and stability, and affects the gating behavior. An effective treatment for F508-del CF patients requires at least two CFTR modulators: a corrector to increase CFTR levels at the cell surface, and a potentiator to increase the opening frequency of the mutant CFTR channel [2,3]. At the moment only two correctors for the treatment of CF patients bearing the F508del-CFTR mutation have been approved, i.e. lumacaftor (VX-809) and tezacaftor (VX-661), in combination with a potentiator, ivacaftor (VX-770). The therapeutic benefit of these combinations is however still unsatisfactory. There is, therefore, the need of new, more effective correctors [4].

Following a HTS approach, we screened a collection of about 15,000 maximally diverse commercial small-molecule compounds, in two different cell types (FRT and CFBE41o-) stably expressing F508del-CFTR, using high-throughput functional phenotypic assays based on the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP) [5]. This activity yielded some primary hits, belonging to different chemical classes. One of these chemo-types was investigated extensively. Rounds of chemical modifications of the hit and functional evaluation in different secondary assays provided the information to build the Structure-Activity Relationships (SARs) within the class. Hit-to-Lead and Lead-Optimization campaigns led to compounds with high potency and efficacy in rescuing the activity of F508del-CFTR in bronchial epithelial cells from CF patients homozygous for the F508del mutation, as measured by electrophysiological assays. The best correctors showed potency in the low nanomolar range, retaining very good efficacy in the single-digit nanomolar range. Several compounds showed drug-like properties suitable for further development upon evaluation in in vitro DMPK assays. The data generated on the most promising correctors will be presented and discussed.

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

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BURULI ULCER AND THE MTOR PATHWAY: TOTAL SYNTHESIS, STRUCTURE–ACTIVITY AND TARGET ELUCIDATION STUDIES OF MYCOLACTONES

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Mycolactones are a group of complex macrolactones with very interesting cytotoxic, immunosuppressive and analgesic properties. As the exotoxins of the human pathogen Mycobacterium ulcerans, mycolactones are central to the pathogenesis of the neglected disease Buruli ulcer, a severe and chronic medical condition characterized by extensive necrotic skin ulcers. Mycolactone A/B, the most biologically active member of the mycolactone family of polyketides naturally occurs as a 2:3 mixture of the cis- and the trans-isomer at the $\Delta^{4',5'}$ double bond of the lower pentaenoate side chain.[1] However, despite extensive research in several academic laboratories, it is not yet clear which of these isomers represents the major contributor to bioactivity. Moreover, the molecular mechanisms of mycolactones action are heavily debated but none of the targets proposed in the previous literature was rigorously validated.

Intrigued by the biological activities of mycolactones, we synthesized a variety of analogs by total synthesis.[2] These compounds featuring modifications at the lower side chain (R$_2$) and the upper core extension (R$_1$) were used to derive essential structure–activity relationships. With the aim of identifying novel druggable targets, biotinylated mycolactones were prepared for target deconvolution studies. By using these tagged probes in conjunction with qPCR, RNAi and immunoblotting, we identified the mechanistic Target of Rapamycin (mTOR) signaling pathway as the key driver of mycolactone action.[3] We showed that mycolactone A/B targets the 12 kDa FK506-binding protein (FKBP12) and interferes with the assembly of the mTORC2 multiprotein signaling complex thereby preventing the activation of the downstream protein kinase Akt. The resulting dephosphorylation of the Akt-targeted transcription factor Forkhead box O3 (FoxO3) triggers the expression of the pro-apoptotic Bcl-2-like protein 11 (Bim) driving cells into apoptosis. Bim knockout protected cells from mycolactone toxicity in vitro and prevented the Buruli ulcer phenotype in M. ulcerans-infected mice confirming our results in vivo. Very recently, we prepared rigidified mycolactone analogs for elucidating the influence of the $\Delta^{4',5'}$-cis/trans isomerism on bioactivity. The synthesis and the SAR of these analogs will also be presented.

References
Tuberculosis (TB) is the curable disease that continues to kill, fuelled by the recent increase in multi-drug resistant infections.[1] In response to the urgent need to combat the rise of resistant infections, the novel diarylquinoline drug Bedaquiline (BDQ) received accelerated approval from the FDA in 2012. Despite being highly effective against drug-resistant TB as a result of its unique mode of action (inhibition of mycobacterial ATP-synthase),[2,3] BDQ has been associated with significant toxicities and issues (hERG mediated cardiotoxicity, phospholipidosis, long half-life) and as such, safety concerns are limiting its clinical use.[1]

The key objective of this project was to synthesise novel and distinct analogues of BDQ with modified structural features, designed to retain high potency whilst improving the safety profile and limiting current side effects. To date, a series of analogues have been synthesised and examined for their activity.

This presentation will outline the development of the synthetic pathways utilised to access these analogues, with our initial focus being on the replacement of the quinoline core. The latest results on the activity of these modified BDQ analogues will also be presented.
SYNTHESIS AND CHARACTERISATION OF PSORALEN DERIVATES AS INHIBITORS OF THE β5i SUBUNIT OF THE IMMUNOPROTEASOME

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The eukaryotic 26S proteasome represents the heart of the ubiquitin-proteasome system. The system is responsible for maintaining protein homeostasis and regulation of many cellular processes, such as antigen processing, signal transduction, cell differentiation and apoptosis. Its 20S core particle has three enzymatically active subunits which have distinct substrate specificities. The β5i (chymotrypsin-like) subunit prefers neutral, hydrophobic residues at the cleavage site and β5i-selective compounds are investigated for possible application in autoimmune and inflammatory diseases related to the immunoproteasome. The majority of currently available inhibitors have a peptidic backbone which makes them prone to poor metabolic stability and low bioavailability.

Previous studies established psoralen derivates with an oxathiazolone 'warhead' as nonpeptidic covalent inhibitors of the β5i subunit.1 With the intent to deepen structure-activity relationship knowledge for psoralens, we synthesised a series of compounds with variations at the R1 position on the parent psoralen. Interestingly, despite seemingly straightforward reactions, several difficulties arose during preparation of some derivatives with substitutions at the R1 position. Our focus was also devoted to the replacement of the oxathiazolone 'warhead'. We introduced succinimide esters, nitrile-based electrophiles, acrylamides, aldehydes, boronates, epoxyketones and ketoaldehydes, while maintaining phenyl as the substituent at site R1. All successfully prepared psoralens were characterised in in vitro and cell-based assays to assess their selectivity and potency.

References

FRAGMENT-BASED APPROACH APPLIED TO THE DISCOVERY OF PROTEIN-PROTEIN INTERACTION STABILISERS

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Protein-protein interactions (PPIs) are constituents of numerous biological pathways and offer therapeutic intervention points into different pathologies such as cancer¹, inflammation², neurodegenerative³ and metabolic diseases⁴.

Since stabilisation of PPIs has not yet been explored in a systematic way, the TASPPI (TArgeted small-molecule Stabilisation of Protein-Protein Interactions) consortium⁵ aims to identify chemical PPI stabilisers in order to develop new crucial therapeutic strategies in the treatment of the disease areas mentioned above.

The Taros fragment collection was selected as the compound source for developing small molecules able to stabilise the complexes of 14-3-3 protein and its partners. The collection is Ro3-compliant⁶ and three-dimensionality and shape diversity have been emphasized as design parameters during the generation process of the library.

The physicochemical properties distribution of the fragment set (Figure 1, a-d) will be presented together with selected examples of novel structures originating from the proprietary collection of Taros.

The design was inspired by two main sources: (i) natural compounds and (ii) known scaffolds from drug discovery campaigns. Nicotine-like fragments (Figure 1, e) represent the perfect match between these two strategies that produced an interesting original Biocore⁷ – the 1,3,5-trisubstitued triazole – and showcases a new concept in fragment design named “SAR by Biocores”. To date, the fragment collection comprises approx. 1.100 fragments and offers ample opportunities for expansion.

Fluorescence polarization, differential scanning fluorimetry, X-ray crystallography and NMR-based techniques have been applied by the consortium members during the primary screening and led to the identification of novel hits binding to different 14-3-3 complexes. These novel binders represent an important starting point for future medicinal chemistry-based fragment evolution campaigns.

Figure 1. a-d) Physicochemical properties of the Taros fragment collection. a) MW and clogP correlation. b) Distribution of H-bond acceptors/donors and rotatable bonds. c) Polar Surface Area (PSA) distribution. d) Saturation index (fsp3) distribution (0 = completely flat, 1 = highly 3D-dimensional). e) Nicotine-like fragment structural evolution.

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Irreversible ligands have been proven to be useful pharmacological tools in the study of structural and functional features in drug receptor pharmacology of G protein-coupled receptors (GPCRs).

Recent advances in the field, which made it possible to obtain ligand-bound X-ray structures by co-crystallizing GPCRs with covalently bound probes, have been one of the major drivers behind the increased interest in the development of novel irreversible probes targeting GPCRs. Here, we will present our quest to solve the first X-ray structure of the adenosine A1 receptor. This includes our efforts to obtain the first X-ray structure of the adenosine A1 receptor, which was stabilized using DU-172, an irreversible antagonist (Figure 1). Furthermore, we have successfully designed, synthesized and evaluated novel irreversible agonists of the adenosine A1 receptor (Figure 2). Four of these compounds, were shown to possess similar potency and efficacy to the reference high efficacy agonist, NECA, in an assay of ERK1/2 phosphorylation assay and two irreversible agonists demonstrated an ability to stabilize purified, detergent-solubilised adenosine A1 receptors in a ThermoFluor assay to a significantly higher degree than NECA. Thus, these results offer an attractive starting point for a range of experiments including our quest to solve the first active-state X-ray structure of the adenosine A1 receptor.

References
INHIBITORS OF HUMAN SIALYLTRANSFERASES AS NOVEL ANTI-METASTATIC AGENTS

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Upregulation of sialyltransferases (STs), the enzymes responsible for the addition of sialic acid to growing glycoconjugate chains, and the resultant hypersialylation of tumour cell surfaces is an established hallmark of many cancers including lung, breast, ovarian, pancreatic and prostate cancer[1]. The critical role of ST enzymes in tumour cell growth and metastasis, as well as links to multi-drug and radiation resistance, have seen STs emerge as a target for potential antimitastatic cancer treatments. There is also evidence showing down-regulation of some STs in neurological disorders such as Alzheimer’s, schizophrenia, autism, and others – highlighting the need for selective inhibition. While multiple examples of potent ST inhibitors are seen in the literature, several challenges remain before they can proceed to the clinic including improving potency and selectivity, as well as addressing pharmacokinetic issues and synthetic accessibility. Herein, we present computational and synthetic studies towards a new generation of ST inhibitors, based on 1,2,3-triazole-linked compounds.

Computational modelling has also been undertaken using available structures of human STs to gauge potential selectivity for ST8 (altered expression in melanoma and prostate cancer, as well as in neurological disorders) over other ST3 and ST6 subtypes. These studied have revealed structural differences between substrate binding sites in ST subtypes whereupon variation of the nucleoside fragment could enhance selectivity[2].

To synthesise 1,2,3-triazole-linked inhibitors, a click reaction between an α-azidophosphonate and 5′-alkynyl uridine was utilised. Biological testing has been undertaken against various human ST subtypes with promising data observed. Results of the computational modelling, synthesis and biological evaluation of these novel ST inhibitors as potential anti-metastatic agents will be presented.

References
3-OXABICYCLO[4.1.0]HEPTANE: A BIOISOSTERE FOR MORPHOLINE AS A KINASE HINGE BINDING MOIETY

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The N-aryl heteroalkyl moiety, e.g. (1), is ubiquitous in medicinal chemistry. In these systems the co-planar conformation is strongly favoured by interaction of the non-bonding pair of electrons on nitrogen with the aryl pi-system. We recently discovered that cyclic systems containing the cyclopropyl group (4) possess a similar conformational bias and the resulting morpholine isostere delivers benefits to physical properties and novelty with the added advantage of removing an embedded aniline.

We have applied this discovery to a lipid kinase family for which N-aryl morpholines (1) are a common hinge-binding group and make a vital hydrogen bonding interaction to the kinase hinge region. Co-planarity between the two rings is a requirement for activity and thus ring systems which adopt orthogonal conformations such as aryl-tetrahydropyran (2) are ineffective as kinase hinge binding fragments,[1] whereas unsaturated systems (3) are often chemically unstable and hence undesirable. 3-oxabicyclo[4.1.0]heptane (4) was found to adopt the required conformation and is a new example of a stable, saturated carbon linked hinge binding moiety for this family of targets.

Herein we describe predictive DFT calculations, X-ray crystallography (figure 1), NMR studies and biological evaluation of a series of tool compounds to comprehensively investigate the conformational preference of 3-oxabicyclo[4.1.0]heptane when attached to a range of 6-membered heterocyclic rings. This informs the scope and limitations of this fragment as both a kinase hinge binding fragment and a general aryl-morpholine isostere.

Our investigations have also uncovered an interesting shift in the physiochemical properties of the 3-oxabicyclo[4.1.0]heptane fragment compared to morpholine containing compounds, which will be of interest to medicinal chemists who are attempting to modify these properties whilst maintaining high-potency.

[Figure 1: 2-pyridyl tool compound X-ray crystallography structures to investigate the conformational preference of various hinge binding fragments.]

References

DEVELOPMENT OF SMALL-MOLECULE INHIBITORS OF ADIPOSE TRIGLYCERIDE LIPASE (ATGL)

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Adipose Triglyceride Lipase (ATGL) is the first and rate-limiting enzyme in the catalytic cascade of lipolysis.\textsuperscript{[1]} Hence, ATGL is primarily responsible for the mobilization of fatty acids (FAs) from cellular triglyceride stores \textsuperscript{[2]} and in consequence the level of circulating FAs.\textsuperscript{[3]} As high levels of serum FAs are closely linked to the development of non-alcoholic fatty liver disease (NAFLD) and insulin resistance, which further progresses to liver steatosis and type II diabetes, respectively, ATGL represents an interesting pharmacological target. This is strongly supported by the results of ATGL knock out studies in mice, which show an increase in insulin sensitivity.\textsuperscript{[3, 4]}

Recently, we described the first potent inhibitor of murine ATGL, Atglistatin\textregistered\ (IC\textsubscript{50} = 0.7 µM). Treatment with Atglistatin effectively reduces FA mobilization \textit{in vitro} and \textit{in vivo}, which leads to a tremendous increase of insulin sensitivity and resistance against the development of NAFLD in mice fed a high fat diet. Still, mice showed no loss in muscle weight or accumulation of TGs in ectopic tissue such as skeletal muscle, or heart in contrast to ATGL-k.o. mice.\textsuperscript{[4]}

The structure of Atglistatin has been developed from the hit compound (shown in Figure 1) in an intense optimization process and is designed to overcome toxicity and solubility issues while increasing potency. It can be produced in a three-step-synthesis. However, Atglistatin inhibits only murine ATGL. To overcome this issue, we are currently working on further optimization of the lead structure to produce a 2\textsuperscript{nd} generation inhibitor.

\begin{figure}
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\caption{Development of Atglistatin\textregistered}
\end{figure}

References
COVALENT FRAGMENT-BASED DISCOVERY OF NEW MURA INHIBITORS

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UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) is involved in the early steps of peptidoglycan biosynthesis and is essential for bacteria. MurA catalyses the transfer of enolpyruvate from phosphoenolpyruvate to UDP-N-acetylglucosamine. It is well-validated target for antibacterial drug discovery, as it is inhibited by clinically used antibiotic fosfomycin, which forms a covalent adduct with the cysteine residue within the active site of MurA.

The covalent drugs are compounds that can form a covalent bond with nucleophilic amino acid residues of proteins. Covalent inhibitors offer a number of advantages over non-covalent compounds, such as high potency and prolonged duration of action, which may result in lower and less frequent dosing and reduced off-target activity. Furthermore, covalent compounds can even decrease risk for development of resistance, which is extremely important in antibacterial drug discovery.

A covalent fragment library containing a large set of different warheads was assayed against MurA from E. coli and S. aureus. Results showed that the majority of fragments bound covalently to the target. Additionally, for the active fragments IC₅₀ values were determined, followed by detailed enzyme kinetic evaluation that revealed reversible and irreversible mechanism of inhibition. We discovered fragments that inhibit both MurAs in low micromolar and nanomolar concentrations. The best compounds had the similar potency as clinically used MurA inhibitor fosfomycin.

The data presented in this study revealed the reactivity and specificity of various covalent warheads that bind to MurA enzymes. This will allow us to select the appropriate warhead and optimize it to yield covalent inhibitor with sufficient potency and selectivity.

References
RATIONAL DESIGN OF OPTIMIZED ALLOSTERIC EFFECTORS OF CATHEPSINS K AND S BASED ON SUCCINIMIDE-GLYCINATE SCAFFOLD

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Cysteine cathepsins are proteolytic enzymes which are located in endosomal and lysosomal compartments of animal cells where they play a fundamental role in the degradation of extracellular and intracellular proteins. Their activity must be carefully regulated since lack of regulation can lead to various diseases. Some cysteine cathepsins are promising drug targets, including cathepsins K and S. Multiple orthosteric inhibitors which covalently bind into the active sites of cathepsins K and S are being developed as drugs for the treatment of several diseases and a few of them already reached clinical trials, however none of them have yet been approved. An alternative way to regulate enzyme activity is allosteric regulation which is emerging as an important strategy for drug discovery and development. Furthermore, allosteric drugs, usually small molecules, have already been approved for the treatment of several diseases. In comparison to orthosteric drugs allosteric drugs bind to evolutionary less conserved sites, making them more specific, safer and causing less side effects [1, 2].

Cathepsin K is the only cysteine cathepsin known to be allosterically regulated thus far. We recently identified and characterized the compound Su-Gly-OMe (methyl (R)-(2,5-dioxopyrrolidine-3-il)glycinate) as an allosteric effector of cathepsin K. We synthesized this compound via nucleophilic addition of glycine methyl ester on maleimide. Using enzyme kinetics we determined its mode of action which is consistent with allosteric effectors NSC13345 and NSC94914 [3, 4]. We also co-crystallized Su-Gly-OMe with cathepsin K and confirmed that it binds to the same allosteric site, but adapts a novel binding mode (Figure A). We have also shown that Su-Gly-OMe partially inhibits not only cathepsin K but also cathepsin S. Our hypothesis is that it binds to the same site on cathepsin S and that on the basis of structural differences between both sites we can develop compounds specific for each enzyme (Figure A). For this purpose we prepared three one-dimensional libraries of compounds with three sites for diversification on the Su-Gly-OMe scaffold. We synthesized them using different nucleophiles or acceptors and similar reaction conditions as for the synthesis of Su-Gly-OMe (Figure B).

We tested the effects of the synthesized compounds on the activities of cathepsins K and S using synthetic substrates. Furthermore, we also determined the affinities of those compounds which acted as inhibitors. By analyzing the structure activity relationship we will be able to identify the groups contributing to the affinity and specificity of the synthesized compounds towards cathepsins K and/or S. Our final goal is to develop high affinity and specific effectors for each enzyme.

References
Irreversible covalent inhibitors were disfavored until the recent development and approval of kinase inhibitors Afatinib and Ibrutinib.\(^1\) Utilizing an acrylamide as the electrophile, these inhibitors form an irreversible covalent bond with a non-conserved cysteine at their binding site. Protein activity can only be restored by de novo protein synthesis, resulting in a therapeutic effect that could last long after the inhibitor is cleared from the blood. Acrylamide moieties also form irreversible covalent bonds with non-targeted thiol residues, and the safety profile of irreversible inhibitors could be improved with the use of latent electrophiles such as terminal alkynes.

Terminal alkynes are generally considered ‘inert’ towards cellular components, and are therefore often used in bioorthogonal approaches as chemoselective ‘Click’ handles. However, in our group it was shown that a propargyl moiety on the C-terminus of Ubiquitin reacts in an activity-based manner with the catalytic cysteine residue in DUBs (DeUbiquitinating enzymes).\(^2\) The lack of indiscriminate reactivity with thiol residues in non-targeted proteins or with excess thiols suggested a proximity-driven reactivity. Utilizing the alkyne moiety in a small molecule inhibitor could thus reduce adverse effects resulting from covalent off-target interactions.

We introduced propargyl derivatives onto the scaffold of Odanacatib (ODN), a selective inhibitor of Cathepsin K (CatK).\(^3\) CatK is one of the most important cysteine proteases in bone degradation, whose aberrant activity has been implicated in diseases such as osteoporosis, osteoarthritis, bone metastasis and giant cell tumor of the bone. The alkyne moiety was positioned to be in close proximity of the catalytic cysteine residue on the active site of CatK, utilizing the alkyne moiety as a latent electrophile. Evaluation of the biochemical properties and inhibitory activity revealed that the compounds inhibit CatK activity with high selectivity compared to other Cathepsins. Inhibition of CatK activity was found to be irreversible, in activity assay on recombinant enzyme as well as in a functional bone resorption assay with human osteoclasts. Intact protein MS confirmed the formation of a covalent inhibitor-CatK complex. Further evaluation of the biological implications is ongoing.

References
EXPLORATION OF CHEMICAL SPACE OF TWO CLASSES OF INHA INHIBITORS

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Tuberculosis (TB) is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, 6.3 million new cases of TB were reported, which is an increase from 6.1 million in 2015. Drug-resistant TB is also a continuing threat and such infections can significantly complicate the treatment. Despite the progress in the pipeline for new diagnostics, drugs, and treatment regimens, there is an urgent need for novel agents.1

*Mtb* enoyl acyl carrier protein reductase (InhA) is an NADH-dependent enzyme that facilitates the reduction of long-chain *trans*-2-enoyl-acyl carrier protein fatty acids. It is a key component of the *Mtb* FAS II pathway and widely recognized as a validated drug target. The initial discovery of two compound classes as direct InhA inhibitors, which represent the foundation for this work, was made by GlaxoSmithKline through a high-throughput screening campaign. These classes are the thiadiazoles2 (represented by the general structure 1, Figure 1) and the tetrahydropyran derivatives (such as compound 2). During the EU-funded 7th Framework Project ORCHID, the structure-activity relationship (SAR) studies of both series of compounds were undertaken. These efforts culminated in the compound *GSK693*3 and a series of pyridinyl-2-thiadiazole based inhibitors of InhA (such as compound 3). In the latter case, we reduced the aromatic ring count of compounds with the aim to improve their physicochemical properties, while retaining their InhA inhibitory potency and antimycobacterial activity. Several other strategies to explore the chemical space of these direct inhibitors were attempted, such as substitution of the thiadiazole central core with other heterocycles, yielding extensive SAR data.

Because of the realization of the need for new antimycobacterial compounds, the work in this field is continuing after the formal completion of the EU-funded project. Our current focus is devoted into further chemical space exploration and SAR definition of InhA inhibitors; i.e by scaffold merging approach (combining the pharmacophores of both compound classes into a single molecule).

![Figure 1. Schematic representation of the studies of direct InhA inhibitors.](https://example.com/figure1.png)

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**References**

The Late-Stage Functionalization (LSF) of peptides has emerged as a valuable strategy for the design of potent peptide-pharmaceuticals enabling rapid exploration of Structure-Activity Relationships (SAR).[1] Furthermore, LSF offers novel opportunities for the introduction of conjugation handles thus allowing for the generation of biological tools as well as peptide-drug conjugates.[2] However, commonly employed methods for the site-selective modification of complex unprotected peptides currently rely on the use of either non-natural amino acids or on the innate reactivity of a very limited number of natural residues (mainly Cysteine and Lysine).[3] Herein we report novel methods for the diversification of peptides under mild reaction conditions. Using this technology, versatile handles were introduced for the tagging and bioconjugation of peptide pharmaceuticals.

Figure 1:

References
IDENTIFICATION OF A GSK-3β/CK-1δ INHIBITOR: DUAL TARGET AND DUAL MECHANISMS OF ACTION AS POSSIBLE STRATEGY FOR THE TREATMENT OF PARKINSON’S DISEASE

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GSK-3β and CK-1δ are emerging targets for the treatment of neuroinflammation-related disorders, including the neurodegenerative Parkinson’s disease. [1-4] In order to address the effect of a contemporary inhibition of the two kinases as a new strategy to counteract neuroinflammation in these disorders, a dual kinase inhibitor was herein developed. We identified a [1,2,4]triazolo[1,5-a][1,3,5]triazine derivative which showed a balanced inhibitory activity on GSK-3β and CK-1δ [IC₅₀(GSK-3β)= 0.17 μM; IC₅₀(CK-1δ)= 0.68 μM]. In particular, a classical ATP-competition was observed against CK-1δ, while a co-crystal of compound inside GSK-3β confirmed a covalent interaction with Cys199. Docking studies were performed on both enzymes in order to better rationalize the obtained results. The developed [1,2,4]triazolo[1,5-a][1,3,5]triazine derivative is not cytotoxic and it is able to prevent MPTP and 6-OHDA mediated cell death in the PC12 cell line, supporting GSK-3β/CK-1δ dual inhibition as a possible new strategy for the treatment of Parkinson’s disease.

References
DISCOVERY OF BENZOTHIAZOLE-BASED DNA GYRASE AND TOPOISOMERASE IV INHIBITORS WITH BROAD SPECTRUM ANTIBACTERIAL ACTIVITY

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DNA gyrase and topoisomerase IV are validated targets for discovery of antibacterial drugs. They are heterotetrameric enzymes composed of two catalytic GyrA/ParC subunits and two GyrB/ParE subunits with ATPase activity. The latter have become attractive targets in many drug discovery projects in pharmaceutical industry, especially after successful introduction of the novobiocin into the therapy. However, novobiocin was later withdrawn from the clinic due to its toxicity and development of bacterial resistance. In recent decades, several new small-molecule GyrB and ParE inhibitors have been discovered. Nevertheless, none of them have advanced beyond Phase I clinical trials, and most of them are only active against Gram-positive bacteria.

Recently, we have discovered and optimized several structural classes of potent DNA gyrase and topoisomerase IV inhibitors with activity mainly against Gram-positive pathogens.1-5 Our latest optimization efforts resulted in the benzothiazole class of potent dual DNA gyrase and topoisomerase IV inhibitors with activities in the low nanomolar range (1-20 nM) against GyrB, which is the primary target of compounds in bacteria. The most potent compounds possess antibacterial activity with MIC values lower than 1 µg/mL against many Gram-positive strains (e.g. Staphylococcus aureus, methicillin-resistant S. aureus, Enterococcus faecalis) and low µg/mL values against Gram-negative strains (e.g. Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, Pseudomonas aeruginosa). The best compounds display activity also against plasmid-mediated quinolone resistant E. coli strains, therefore, showing no cross-resistance with the fluoroquinolones. In addition, resistance potential in E. coli was determined and mutations were mapped to the residues in the ATPase domain of GyrB. Further in-depth studies are currently in progress to reveal true potential of the most advanced compounds as potential antibacterial agents.

References
DEVELOPMENT OF PEPTIDES AS THERAPEUTICS FOR PHARMACOLOGICAL INTERVENTION IN VITAL PROTEIN CASCADES

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As a therapeutic molecule class, peptides combine advantages of protein-based biomolecules, such as high affinity, specificity and ability to target “undruggable” targets, with the easy synthetic accessibility and chemical modification of small molecules. Cyclic peptides in particular can utilize a high surface area for binding, which makes them suitable for targeting protein-protein interactions. With their comparatively constrained structures, they are reducing the entropic penalty upon binding and their rigidity renders cyclic peptides more resistant to proteolytic degradation than their linear counterparts. Several cyclization methods are used, such as on-resin side specific lactamization, disulfide bridging, thioether cyclization or usage of thiol-reactive linker.

In this study, we describe the development of phage display-derived cyclic peptides for the therapeutic modulation of vital protein cascades within the blood circulation. Both the complement system and the coagulation cascade serve as “first line of defense” against injurious stimuli and microbial invaders: upon activation, a series of cascading enzymatic reactions lead to an amplification of the initial signal, resulting in fibrin deposition (coagulation cascade), pathogen clearance and opsonic cell killing (complement cascade). In several thrombo-inflammatory conditions, including transplant rejection, stroke and reperfusion injury, both host defense systems may be inadvertently triggered and contribute to clinical complications. Therapeutic control of complement and coagulation activation has therefore gained attention.

For example, blocking coagulation factor XII (FXII) has been shown to reduce thrombosis in various animal models without increasing the risk for bleeding, a major problem of current anti-coagulants. Moreover, plasma kallikrein (PK) amplifies FXII activity and is also considered an important target due to the generation of proinflammatory kinins. By employing a bicyclic peptide phage display approach with on-phage chemical cyclization, we obtained bispecific FXII/PK inhibitors, which were improved to inhibit both targets in the nanomolar range using additional structure-activity relationship studies.

A similar approach can also be applied to the development of complement inhibitors. Cyclic peptides have shown great promise as protein-protein interaction inhibitors in the complement cascade. Through incorporation of unnatural amino acids and other modifications, we aim to improve affinity, selectivity and pharmacokinetic properties of such leads for a use in a broad range of disease models.

References
Contemporary medical practice is elevating the need for antibacterial drugs and with it, an imminent upsurge of bacterial resistance is observed. In lieu of diminished effectiveness of antibacterials in materia medica, identification of novel antibacterial compounds serves as a crucial topic for research investment. Deep learning as a selection of machine learning techniques can produce high level abstractions from large and heterogeneous data sets of high-dimensions and methodology is suitable for composite property predictions. Last-mentioned input data set was collected from expanding ChEMBL v23 database where pruned libraries of antibacterial compounds were constructed. Using Keras neural networks Python API and multiple bioinformatics software packages, molecular fingerprints and descriptors were calculated and served as input data for training, testing and optimization of deep neural network models. Constructed models were able to identify compounds with or without antibacterial activity against Gram-positive (S. aureus) or Gram-negative (E. coli) bacteria with higher accuracy when compared to several alternative or classical QSAR approaches. We were also able to prospectively study the antibacterial properties of in-house databases and confirm the results with in vitro antimicrobial evaluation on relevant bacterial strains. Furthermore, we postulate the application of reported methodology for library enrichment and antibacterial compound design.

References
THE DESIGN AND SYNTHESIS OF NOVEL INHIBITORS OF THE NOTCH ACTIVATION COMPLEX KINASE FOR NOTCH MEDIATED TUMORIGENESIS

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Notch Activation Complex Kinase (NACK) was recently identified as a key player in Notch-mediated tumorigenesis by Capobianco et al\textsuperscript{1}, fashioning NACK as an attractive novel target for the treatment of esophageal adenocarcinoma. However, there is no co-crystal protein ligand structure in the Protein Data Bank and no reported biological data, marking NACK as an understudied target. In order to identify novel scaffolds for NACK inhibition, a machine learning approach was employed, where over 6 million commercially available compounds were screened against established kinase machine learning classifiers, and nearly 8000 were prioritized based on the predicted probability of being active. A structure model of the NACK kinase domain was generated through homology modeling and further optimized with molecular dynamics (MD) simulations, followed by virtual screening of pre-prioritized compounds. Top-scoring compounds were purchased and screened in \textit{in-vitro} and \textit{in-vivo} assays. Commercially available compound Z271-0326 (iNACK) displayed the best inhibitory activity and was further validated in a xenograft mouse models. A robust novel chemical synthesis for iNACK which was accomplished in six steps with an overall yield of 26%. A small library of diverse analogues were synthesized, and assayed to further probe iNACK’s mode of binding. Using this information, we then optimized the NACK kinase domain structure model via extended molecular dynamics studies with known active and inactive compounds to better understand binding interactions, and increase model stability. We are continuing to optimize iNACK into the first NACK molecular probe and advanced pre-clinical lead by utilizing computationally-driven structure-activity relationship (SAR) studies to improve binding affinity and inhibitory activity, as well as using interactive optimization platforms to increase affinity and favorable ADMET properties.

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Posters Presentations
MECHANISM OF WATER TRANSPORT THROUGH SGLT1 PROTEIN

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SGLT1 is a membrane protein and a high-affinity Na+/glucose cotransporter that is involved in the transport of glucose and galactose across the luminal side of enterocytes in the small intestine (1). In addition to its capacity to transport substrates this protein also facilitates the movement of water across the cell membrane. Using explicit molecular dynamics simulations of SGLT1 and two of its mutants - F453C and Q457C in addition to a double mutant (2), we account for the systematic coupling between the water movement and local protein dynamics. We calculate various time dependent parameters describing local structure of the water channels, including thermodynamic, electrostatic and geometric aspects. We determine the correlations between these parameters, with the final goal of better elucidating the mechanism of water transport in SGLT1.

References

FLOW PHOTOCHEMICAL SYNTHESIS OF THE FUNCTIONALIZED POTENTIALLY BIOACTIVE BENZOBICYCLO[3.2.1]OCTADIENE SKELETON

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Compounds with the bicyclo[3.2.1]-skeleton have been proven as potent inhibitors of dopamine and serotonin transporters and they also play a crucial role in treatment of CNS and Alzheimer’s diseases [1]. By utilizing photochemical synthesis a whole library of new polycyclic compounds with the benzobicyclo[3.2.1]octadiene moiety was obtained [2]. For the first time, flow photochemistry was applied as an even more powerful tool [3] for the synthesis of the cycloadducts 1-5 bearing this crucial bicyclo[3.2.1]-core. In comparison to the batch reaction, the flow reaction showed better results, both increasing the isolated yields and productivity and in lowering the reaction time.

For all of the obtained novel potentially bioactive derivatives preliminary physico-chemical properties were also analyzed and the measurements of cholinesterases inhibitory activity (enzymes important at different stages of Alzheimer’s disease). Among them compound 3 showed the best acetylcholinesterase and butyrylcholinesterase inhibitory activity [4].

References
NOVEL ADAMANTYL 3-HYDROXYPYRIDIN-4-ONES: SYNTHESIS AND ANTIPROLIFERATIVE IN VITRO STUDY

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3-Hydroxypyridin-4-ones are investigated for their broad spectrum of biological activities (antibacterial, antineurodegenerative, anticancer).[1] Recently, we investigated N-aryl substituted 3-hydroxy-2-methylpyridin-4-ones as well as their adamantyl derivatives for their in vitro antitumor properties on several cancer cell lines.[2] All tested compounds showed activity ranging from moderate to strong on all cell lines with adamantane containing derivatives being active at low micromolar IC50 concentrations. Further structure-activity relationship study (SAR) of such and similar pyridinone derivatives as potential anticancer agents is in progress. In this work, for the purpose of that study, novel adamantyl derivatives of N-aryl substituted 3-hydroxy-2-methylpyridin-4-ones were prepared with the aim of evaluating their in vitro antitumor properties on the panel of cancer cell lines. The compounds were synthesized starting from corresponding pyridinones, which are prepared first in an autoclave, and adamantane-1-ylacetic acid. Antitumor properties of novel compounds will start to elucidate the key elements, primarily the position of adamantyl unit, needed for high activity of observed pyridinone derivatives. In the case of high antiproliferative activities of novel derivatives in vitro testing will include cytotoxicity evaluation and additional testing of the most potent candidates to determine the mechanism of their action.

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References
DESIGN, SYNTHESIS AND EVALUATION OF TOLL-LIKE RECEPTOR 7 AGONISTS WITH 2-(TRIFLUOROMETHYL)QUINOLINE-4-AMINE AND 2-(TRIFLUOROMETHYL)QUINAZOLINE-4-AMINE SCAFFOLD

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Toll-like receptors (TLRs) are pattern-recognition receptors that play an important role in the innate immune responses against a number of pathogens. TLR7, one of the 12 functional TLRs discovered up to date, is recognized as a promising target for the treatment of viral infections, autoimmune diseases and cancer. For identification of potential novel ligands of TLR7 our ligand-based virtual screening protocol named LiSiCA was used, with imiquimod as a query compound. 22 compounds, topologically most similar to the reference compound, were obtained from different vendors. After biological evaluation of their agonist activity two hit compounds with similar scaffolds, namely 2-(trifluoromethyl)quinoline-4-amine and 2-(trifluoromethyl)quinazolin-4-amine, were discovered. Concurrently, a simple three-step synthetic procedure was developed to resynthesize initial hits and prepare a focused library of their analogs. 22 novel compounds were synthesized and evaluated for TLR7 agonist activity on the HEK293 cell line, co-transfected with hTLR7 gene and an inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Activation of hTLR7 receptors triggers higher secretion of SEAP, which could be measured colorimetrically in the supernatant using Quanti-Blue reagent. EC50 values of the most potent agonists were determined in the micromolar range, with the most potent one of 53.1 µM. All active compounds were further tested on HEK293-hTLR8 cells using the same assay protocol. None of our TLR7 agonists showed any activity on TLR8. Even though our compounds are less potent TLR7 agonists compared to imiquimod, they show selectivity toward TLR7, thus representing an important starting point for further studies of small-molecule agonists with novel 2-(trifluoromethyl)quinoline-4-amine and 2-(trifluoromethyl)quinazolin-4-amine scaffolds.

References
Protein kinase C (PKC) isoforms regulate numerous cellular functions, making them highly attractive drug targets.\(^1\) Utilizing the crystal structure of the PKC\(\delta\) C1B domain,\(^2\) we have developed hydrophobic isophthalic acid derivatives which allosterically modulate PKC activity by targeting the C1 domain of the enzyme.\(^3, 4\) In the present study,\(^5\) we aimed to improve the drug-like properties of the isophthalic acid derivatives by increasing their solubility and enhancing the binding affinity. We synthesized a series of multisubstituted pyrimidines as analogs of C1 domain–targeted isophthalates and characterized their binding affinities to the PKC\(\alpha\) isoform. In contrast to our computational predictions, the scaffold hopping from phenyl to pyrimidine core diminished the binding affinity. However, the present results provide useful structure-activity relationship data for further development of ligands targeted to the C1 domain of PKC.

References

INVESTIGATION OF A NEW COLON-TARGETED ANTIBIOTIC CLASS FOR THE TREATMENT OF INFECTIONS CAUSED BY GASTROINTESTINAL PATHOGENS

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Common inhabitants of human gut microflora can cause deadly disease upon opportunistic colonization of the gastrointestinal tract. Two bacteria of clinical importance are Clostridium difficile and Enterococcus faecium which can cause infections that become difficult to treat and a significant threat to human health. The spores produced by C. difficile are not able to be fully eradicated following treatment and cause a relapse of infection in a significant number of patients. In the cases of E. faecium infections, the inherent ability to form drug-resistance is seeing the emergence of many strains displaying decreased susceptibility to even the last-line treatments of Linezolid and Daptomycin. (1, 2)

Recently a novel antibiotic drug class of 1,2,4-oxadiazole compounds has been discovered that exhibit considerable activity against several Gram-positive pathogens. (3) The class is active against both C. difficile and E. faecium but not against certain Gram-negative pathogens which demonstrates the potential for selective bacterial targeting in the gastrointestinal tract. The oxadiazole compounds were modified in an effort to develop orally efficacious antibiotic agents for treating infections in the colon. The development of this new class of colon-targeted antibiotics advances progress toward the decolonisation of gastrointestinal pathogens as well as effective treatment of the life-threatening infections they cause.

References

ANTIMICROBIAL ACTIVITY OF QUINUCLIDINE BASED CATIONIC SURFACTANTS AGAINST LISTERIA MONOCYTOGENES

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Listeria monocytogenes is a rapidly growing Gram-positive food pathogen that causes listeriosis in humans which, if untreated, can lead to serious and often fatal consequences. Once L. monocytogenes are organized in biofilm structures, they are more resistant to antibiotics and as such pose a great challenge to scientists 1.

One of the most powerful antimicrobial agents are quaternary ammonium compounds (QAC) that are widely used as antiseptics in homes and hospitals worldwide. As amphiphilic compounds, these agents interact with negative bacterial membranes causing an osmotic imbalance that subsequently leads to perforation of the cell wall structure. For this reason, QAC are considered as agents of a broad spectrum antibacterial activity. However, a worrisome number of bacterial strains isolated from the environment have become resistant to commercial QAC highlighting the importance of new drug discoveries 2. An underexplored natural QAC that has promising medicinal and pharmaceutical potential is a quinuclidine isolated from the cinchona tree. Our group has previously shown that quinuclidine based QAC have good antibacterial potential, especially toward Gram-positive bacterial strains 3. Motivated by these observations, we were interested whether QAC consisting of a quinuclidine core and long side alkyl chains (C12 and C14) exhibit antimicrobial activity against L. monocytogenes and whether these compounds have an effect on a biofilm formation.

Our results indicate that quinuclidine derived QAC have a promising antimicrobial activity against L. monocytogenes with MIC and MBC values of up to 3.9 µg mL⁻¹. An upcoming research on the fractional inhibitory concentration and antimicrobial activity against biofilm structures will showcase its benefit in manifold applications.

References
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL ADAMANTYLTHIOUREA, ISOTHIOUREA AND RELATED DERIVATIVES

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The incorporation of an adamantyl moiety into several molecules results in compounds with relatively high lipophilicity, which in turn can modify the biological availability of these molecules. Beyond increasing partition coefficients, the adamantyl group positively modulates the therapeutic index of many experimental compounds, through a variety of mechanisms. Several adamantane derivatives have long been known for their diverse biological properties, mainly as antiviral, antibacterial, and antifungal. In addition, thiourea and isothiourea nucleus were reported to constitute the pharmacologically active moiety of several compounds. On these bases, new series of 1-adamantyl derivatives, in which the adamantyl moiety was covalently conjugated to arylthiourea or 4-Thiazolidinone moieties have been synthesized as potential bioactive agents.

The reaction of 1-adamantylamine with various aryl isothiocyanates yielded the corresponding 1-(adamantan-1-yl)-3-arylthiourea derivatives (A). The reaction of the thioureas (A) with various arylmethyl bromides and ethyl bromoacetate, in acetone, in the presence of potassium carbonate yielded the corresponding (Z)-3-(adamantan-1-yl)-1-aryl-S-(benzyl or substituted benzyl)-isothioureas (B) and ethyl 2-[(Z)-1-(adamantan-1-yl)-3-arylisothioureido]acetates (C). The 3-(adamantan-1-yl)-2-aryliminothiazolidin-4-ones (D) were obtained by cyclization of the ethyl 2-[(Z)-1-(adamantan-1-yl)-3-arylisothioureido]acetates (C) via prolonged heating with sodium acetate in ethanol. The structures of the compounds (A-D) were confirmed by analytical and spectral data and single crystal X-ray diffraction. Compounds (A-D) were tested for in vitro activities against a panel of Gram-positive and Gram-negative bacteria and the yeast-like pathogenic fungus Candida albicans, several derivatives produced good or moderate activities particularly against tested Gram-positive bacteria.

In the present investigation, 27 new target compounds were prepared, 15 of them displayed potent antibacterial activity. The purity of the newly synthesized compounds was checked by (TLC), and the structures of these compounds were confirmed by 1H NMR, 13C NMR, (EI-HRMS) or (ESI-MS). In addition, the stereochemistry of some of the compounds that were synthesized were studied by X-ray crystallography.
DESIGN AND PHARMACOCHEMICAL EVALUATION OF NOVEL SUBSTITUTED-CINNAMATE AND COUMARIN DERIVATIVES AS PLEIOTROPIC AGENTS

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Recently, intensive research has been conducted on cinnamic acid scaffold, seeking to create new polyfunctional drugs acting as inhibitors on multiple biological targets. [1] Substituted cinnamic acid hybrids as well as natural coumarinyl derivatives exhibit a wide range of biological activities whereas hybrids combining both scaffold are used as drugs with anticoagulant, anti-inflammatory, antimicrobial, antioxidant and anticancer properties [2,3]. In our laboratory the last decade several derivatives of cinnamic acids have been designed and synthesized as potent pleiotropic agents e.g. lipoxygenase inhibitors, antioxidants and anti-inflammatories.

In continuation of our research, we made an attempt to design and synthesize two series of new multitarget agents cinnamic acid-based drug candidates: a) hybrids of substituted cinnamic acids with known drugs and drug-like molecules, such as paracetamol, hymecromone, propranolol, atenolol, 7- or 4- or 6-OH-coumarin, and b) acetic acid derivatives of 6- and 7-hydroxycoumarins with several amines. [4]

For the synthesis of the novel hybrid compounds we applied known synthetic procedures and simple techniques. The compounds have been identified using spectroscopic methods and they were tested in vitro: a) as antioxidant and scavenging agents, b) as inhibitors of multiple biological targets implicated in inflammation e.g. lipoxygenase, trypsin.

References
DESIGN AND SYNTHESIS OF "THREE-VECTORS" CYCLOPHILINS BINDERS

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Cyclophilins are folding helper enzymes belonging to the class of peptidyl prolyl cis-trans isomerases. They catalyze the cis-trans isomerization of peptidyl prolyl bonds in unfolded and native proteins, playing a pivotal role in a multitude of cellular functions like cell growth, proliferation, and motility. The selective inhibition of these enzymes by different ligands could represent a compelling strategy for the treatment of various pathologies, such as viral infections, neurodegenerative disorders, and cancers, providing a better understanding of the physiological role of the various cyclophilins in the human body.

We have setup a platform that combines computational analyses, organic synthesis, structural studies, biophysical and in vitro assays to understand how existing ligands interact with different cyclophilins. Our overall objective is to delineate design principles for isoform-selective cyclophilin inhibition, and to develop new cyclophilin ligands with auspicious binding affinity and selectivity profiles.

Following a ‘three-vector’ strategy, our aim was to generate a molecule able to establish interactions with two conserved Abu & Pro pockets, and a less conserved remote 3o’clock pocket (See Scheme). Combining MD simulations and chemical syntheses, this novel and unique binding mode to Cyps has been translated into a new class of compounds with promising binding and selectivity profile on Cyps, paving the way for the development of more potent and isoform-selective ligands.

Scheme:

References
BENEFICIAL EFFECTS OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS ON VASCULAR DYSFUNCTION

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Endothelial dysfunction that may result from multiple factors including loss of balance between vasoconstrictors and vasodilators, oxidative stress, inflammation, dysfunctional immunity, dyslipidemia and hyperglycemia, alters vascular homeostasis and contributes to progression of vasculopathies and complications to a wide spectrum of disorders and organ damage. Endothelial cells show significant expression of dipeptidyl peptidase-4, besides its soluble circulating form. Inhibition of dipeptidyl peptidase-4 might participate in preservation of endothelial function, its integrity and vasculoprotection. Mechanisms underlying beneficial effects of dipeptidyl peptidase-4 inhibitors on vascular dysfunction are ascribed to its catalytic and receptor-like activity, improvement of glyco- and lipometabolic profiles, impacts on mediators of oxidative stress, apoptotic markers, inflammatory signaling, number and mobilization of endothelial progenitor cells, vascular smooth muscle cells proliferation and vascular tone.

We pointed to beneficial effects of dipeptidyl peptidase-4 inhibitors in the repair after myocardial infarction by the prevention of the cleavage of chemoattractant cytokine stromal cell-derived factor-1,1 and this work represents the continuation with the aim to gain more detailed insight into multiple favorable effects of dipeptidyl peptidase-4 inhibition in the improvement of vascular dysfunction.

References
SELECTIVITY OF PYRAZOLOQUINOLINONE DERIVATIVES TOWARDS THE ALPHA1+/BETA1- INTERFACE OF THE GABAA RECEPTOR

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GABA receptors are the major inhibitory neurotransmitter receptors in the central nervous system. These GABA-gated chloride channels are composed of five subunits that can belong to different subunit classes. Several pyrazoloquinolinone ligands have already been described as high affinity ligands of the benzodiazepine (Bz) binding site but also, they exert a positive modulatory effect at the a+b- interfaces.1,2 Previously, it was shown that some pyrazoloquinolinone derivatives showed preference towards b1 containing receptors in terms of potency. Further studies in homology models and mutant receptors confirm that the amino acid located in position 41 of segment G in the b1 and b3 subunits strongly influences the potency and efficacy of the tested ligands.3 In the present study, further pyrazoloquinolinone derivatives were studied and results showed that they possess improved functional selectivity. The results of this study are herein presented and the properties of these compounds will be further investigated.

References

Candida auris is a diploid yeast and human fungal pathogen first documented in 2009, in Japan. This emergent species, though new, poses a great risk to human health owing to its extensive drug-resistance profile, and high mortality rates (~30-60%).

Previous work by our group has identified acetohydroxyacid synthase (AHAS), an enzyme responsible for de novo synthesis of branched-chain amino acids (BCAA’s) and currently used as a target for many commercial herbicides, as a viable target for anti-fungal drug development. Here we have shown that commercially available herbicides in the sulfonylurea and triazolopyrimidine family can act as potent inhibitors of a drug-susceptible and drug-resistant strain of C. auris, with MIC50’s as low as 97 nM.

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IN SILICO STUDIES OF INTERACTIONS OF ALLOSTERIC MODULATORS WITH DOPAMINE D2 RECEPTOR

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Currently one of the hot topics in drug discovery is design of allosteric modulators of GPCRs instead of orthosteric ligands. The allosteric mode of action brings several advantages, e.g. ceiling effect preventing overdosage, high receptor selectivity, and even activation pathway selectivity which may in consequence lead to safer and more efficient drugs.

The aim of our studies was to construct homology models of human D_{LONG} receptor (the isoform including long intracellular loop 3, IL3) in active conformation in complex with G_{i1} or G_{i2} protein and to use these models to investigate their interaction with dopamine and a recently reported D_{2} receptor positive allosteric modulator, PAM (see below) [1]. The studied racemic compound acts as a PAM of the rat and human dopamine D_{2} and D_{3} receptors. The R isomer did not directly stimulate the dopamine D_{2} receptor but potentiated the effects of dopamine. In contrast the S isomer attenuated the effects of the PAM and the effects of dopamine (displayed negative allosteric modulator, NAM properties) [1].

The homology models of D_{LONG} receptor in complex with respective G proteins were built using Modeller applying the X-ray structures of β_{2} adrenergic receptor in complex with G_{s} (PDB ID: 3SN6) as a template for helix bundle and G proteins, as well as X-ray structures of dopamine D_{2}, D_{3} and D_{4} receptors in inactive conformation (PDB ID: 6CM4, 3PBL and 5WIU, respectively) as additional templates. Yasara software was used to generate a long receptor IL3 loop, consisting of 139 residues which was refined using Modeller based on its predicted secondary structure. Dopamine was docked to the receptor models using induced-fit docking approach of Schrödinger software while enantiomers of a modulator were docked using Surflex incorporated in Sybyl.

Molecular dynamics simulations using Gromacs were performed to study the effect of the ligands on the receptor. To properly simulate subtle allosteric effects, emphasis on native-like conditions was put. For this purpose, the active-state models with G proteins were immersed in an asymmetrical membrane composed of 8 types of lipids in proportions appropriate to membrane rafts. Amber force field was used to describe the interactions of protein and ligands while the Slipids were used to describe the cell membrane. The trajectories were analyzed using the Principal Component Analysis and Mutual Information methods.

References
Electrospinning is a simple and versatile technique used for the fabrication of continuous micro and nanofibers. This approach is inexpensive, scalable, reliable and mainly used from polymer solutions and polymer melts.\textsuperscript{[1]} Nonpolymeric molecules can usually not be electrospun, as only polymer solutions or melts are sufficiently viscous to provide the required degree of molecular entanglement.\textsuperscript{[2]} However, recent studies have demonstrated that high molar mass polymers are not essential for production of uniform electrospun fibers but that sufficient intermolecular interactions acting as chain entanglements is the primary criterion.\textsuperscript{[3]} Recently it was demonstrated that the dipeptide phenylalanine-phenylalanine (FF), and two Fmoc derivatives, \textit{i.e.} Fmoc glycine (Fmoc-Gly) and Fmoc-phenylalanyl-glycine (Fmoc-Phe-Gly), in spite of their small size, can assemble by electrospinning to nanofibers basing solely on noncovalent interactions.\textsuperscript{[2,4]} Starting from this observation we focused on the exploitation of sulfur/nitrogen containing heterocycles having particular features that can improve the self-assembling propensity of the system. Several compounds containing natural amino acids (Gly, Ala, Leu, Val) together with a heterocyclic scaffold properly functionalized, were synthesized and a study on their electrospinnability was developed. The compounds were dissolved in high concentration in HFIP and the experiments executed on a <5 ml scale using microliter electrospinning technique and on 0,5 ml scale using a conventional setup in laboratory scale. Interestingly, different behaviors were observed for different stereoisomers of the same compound. Obtained fibers were characterized with different techniques: optical microscopy, SEM, AFM, Raman and FT-IR spectroscopy. The fibers appear continuous, with a diameter between 400 and 700 nm and with a full cross-section demonstrating for the first time the possibility to develop electrospun nanofibers from unnatural small molecules.

References
MOLECULAR DESIGN AIDED BY RANDOM FORESTS AND SYNTHESIS OF POTENT TRYPANOCIDAL AGENT FOR CHAGAS DISEASE TREATMENT


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Cruzain is an established target for the identification of novel trypanocidal agents, but how good are in vitro / in vivo correlations? This work describes the development of a random forests model for the prediction of the bioavailability of cruzain inhibitors that are Trypanosoma cruzi killers. Some common properties that characterize drug-likeness are poorly represented in many established cruzain inhibitors. This correlates with the evidence that many high affinity cruzain inhibitors are not trypanocidal agents against T. cruzi. On the other hand, T. cruzi killers that present typical drug-like characteristics are likely to show better trypanocidal action than those without such characteristics. The model was confirmed using other machine learning methods (artificial neural networks and support vector machines) and validated with the synthesis of new trypanocidal agents designed on the basis outlined in this work - that is, compounds with imprinted bioavailability profile.

References
DESIGN AND SYNTHESIS OF NOVEL BENZOTHIAZOLE-PIPERAZINE PROPA NAMIDES AND THEIR BIOLOGICAL EVALUATION AS ACETYLCHOLINESTERASE INHIBITORS

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Alzheimer’s Disease is a progressive fatal neurodegenerative disorder which is characterized clinically by behavioral and cognitive deterioration. Despite many factors affecting the disease progression such as; beta amyloid accumulation leading senile plaques, neurofibrillary tangles, drugs that meet the cholinergic deficiency are widely used currently for the symptomatic treatment. Previous studies indicate that the core ring system, linker and the basic terminus show a template for promising acetylcholinesterase inhibitors (AChEIs) (Fig. 1). According to recent studies, benzothiazole as the core ring, piperazine derivatives as basic terminus with acetamide linker showed potent AChEI activity and good selectivity against acetylcholinesterase (AChE) rather than Butyrylcholinesterase (BuChE). In this regard, we synthesized five novel benzothiazole-piperazine propanamides and determined their biological activities by modified in vitro spectrophotometric method of Ellman also docking studies were performed on AChE. As a result, dimethylaminoalkyl piperazine derivatives 4 (IC50: 351 nM) and 5 (IC50: 303 nM) were found to be more potent AChE inhibitors than Galantamine (IC50: 740 nM) and showed comparable activity with Donepezil (IC50: 103 nM). In addition, compounds 4 and 5 had higher selectivity over AChE than BuChE as a promising result for design of hit compounds.

References
FINDING NOVEL 14-3-3 PROTEIN-PROTEIN INTERACTION MODULATORS USING DYNAMIC COMBINATORIAL CHEMISTRY

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Protein-Protein Interactions (PPIs) can be found in many biological processes. It is assumed that between 130,000 and 600,000 PPIs exist, some play a role in carcinomas others for example in cell-cycle regulation. The 14-3-3 protein family is known for its PPIs, as it is implicated in several diseases and biological processes.(1) Proteins of this family do not have any enzymatic activity, however, they interact and regulate the activity of other proteins. Finding modulators which could stabilize or inhibit the PPIs, would constitute a tool to modulate these interactions and possibly interfere with undesired biological processes by targeting the corresponding PPIs. Dynamic Combinatorial Chemistry (DCC) is a powerful tool to identify biologically active compounds.(2) The strength of this technique is the amplification of the best binders by the target. We pioneered, DCC for the identification of modulators of 14-3-3 proteins, representing its first application to a PPI. Biochemical evaluation of the amplified hits to confirm the activity and optimization of promising compounds is ongoing.

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Monoamine oxidases are mammalian flavoenzymes responsible for the regulation of amine neurotransmitter levels. These enzymes represent main pharmacological targets for the treatment of depression and neurodegenerative diseases. Two isoform of this enzyme are present in human body, MAO A and MAO B, which share about 70% of the identity in the primary sequence, but show significant differences in substrate selectivity and inhibitor specificity in particular. [1] Focus of this work are selective irreversible inhibitors of MAO B, selegiline and rasagiline, widely used in the treatment of symptoms of Parkinson and Alzheimer disease. Both inhibitors form covalent bond with the organic cofactor flavin adenine dinucleotide (FAD). In that way they prevent MAO B enzyme’s further catalytic activity.

Here, we used molecular dynamics (MD) simulations to simulate 300 ns of the interaction of MAO B with both inhibitors. It is shown that Tyr398 and Tyr435 form aromatic cage responsible for interaction with aromatic part of each inhibitor. Ile199 is identified as structurally responsible for the selectivity of the inhibitor, which confirms the experimentally obtained results. [2] Aromatic interactions of the inhibitors with the aromatic cage amino acids as well as the hydrogen bonds between the inhibitors and the flavin carbonyl oxygen O8 orient the inhibitors in a favorable position for the reaction leading to the covalent binding between FAD and the inhibitor. Using MM-PBSA tools, binding free energies were obtained. The results show that selegiline binds better than rasagiline by 1.4 kcal/mol, which is consistent with experimental IC50 values. [3]

Quantum-chemical analysis within the enzyme cluster model showed that MAO inhibition proceeds through a 4-step reaction, with the first step determining the total reaction rate, in which FAD cleaves the hydride ion from the α-methylene group of the substrate in complete analogy with the MAO catalytic mechanism. [4] The resulting reaction profiles and the final structure of the inhibited enzyme are in excellent agreement with the experimental data.

The obtained results are of a great importance for the development of new and more effective MAO B inhibitors for clinical use.

References
DESIGN AND SYNTHESIS OF NOVEL TRYPTOPHAN-BASED HUMAN BUTYRYLCHOLINESTERASE INHIBITORS

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Butyrylcholinesterase (BChE) is a promising drug target in advanced Alzheimer’s disease, a progressive neurodegenerative disorder. Selective BChE inhibitors could restore reduced cholinergic neurotransmission in patients with Alzheimer’s disease, improve their cognitive functions and the quality of life.1 Screening of (+)-isocampholenic acid derived amide library uncovered tryptophan-based hit compound 1 as a promising inhibitor of human (h)BChE (IC50 = 0.25 μM).2 Encouraged by the initial results, we used structure-based drug design to design, synthesize and biologically evaluate reversible hBChE inhibitors with potencies in low nanomolar range and high selectivity for hBChE versus murine acetylcholinesterase (AChE). Resolved crystal structures of complexes with hBChE suggest a different binding mode than previously reported for piperidine based inhibitors3 and offer possibilities for further optimization of the inhibition.

References
EXPLORING HIT-IDENTIFICATION STRATEGIES FOR ENERGY-COUPING FACTOR TRANSPORTERS, A NOVEL TARGET FOR THE DEVELOPMENT OF ANTIBIOTICS

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The emergence of drug resistance against important pathogens poses an ever-growing health threat. The pipeline of novel drug candidates should be filled with molecules featuring an unprecedented mode of action and an unprecedented chemical structure. We address both challenges by using multiple hit-identification strategies targeting a novel and unexplored anti-infective drug target, called Energy-Coupling Factor (ECF) Transporter. The application of various hit-identification strategies – structure-based design, dynamic combinatorial chemistry, and virtual screening – resulted in the identification of new ligands for the ECF transporters. The ECF module is an integral membrane protein involved in the uptake of essential micronutrients.1 Hence, the inhibition of this transport should translate into a deficiency of vitamins in the bacterial cytosol.

We embarked in a structure based drug design (SBDD) of thiamine analogue as binders of ThiT in order to identify which residues are essential for substrate binding and to elucidate the mechanism of transport.2 In parallel, to enrich the structural diversity of ECF inhibitors, we used Dynamic Combinatorial Chemistry (DCC) to explore the large and partially flexible substrate-binding pocket of the ThiT protein.3 This time we wanted to mimic the natural substrate and a library of differently substituted aldehyde and hydrazide delivered a pool of inhibitors bearing the thiamine core.4

A structure-based virtual screening (SBVS) provided us with the first allosteric inhibitors of the transporter for folate able to both reduce folate concentration in the cytosol and to reduce the bacterium growth. Additionally, the excellent drug-like properties of this chemical class of compounds triggered a medicinal chemistry campaign that turned out with the first inhibitors against the ECF transporters active against a plethora of pathogenic Gram-positive organism (Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium).

References
SILENT AGONISM MEDIATED BY THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR: THE ROLE OF TRIFLUOROMETHYL GROUP IN THE NS6740 MOLECULAR SKELETON

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The hypothesis that states a relationship between non-ionotropic activity of the α7 nicotinic acetylcholine receptor (nAChR) and its desensitized states is an emerging research topic. The ability of a new class of compounds, defined as “silent agonists”, to stabilize the desensitized states of the α7 nicotinic acetylcholine receptor engendering anti-inflammatory responses, most likely via a metabotropic mechanism, would seem to confirm that hypothesis (1).

In this study, we dissected the exemplary compound NS6740 (2) ((1,4-Diazabicyclo[3.2.2]-non-4-yl[5-[3-(trifluoromethyl)phenyl]-2-furanyl]methanone)), the most potent desensitizing agent for the α7 nAChR, characterized by both profound desensitization and relatively long term binding to the receptor. NS6740 shows promising anti-inflammatory activity, both in vitro and in vivo, in a mouse model of chronic pain and inflammation (3,4).

In particular, we explored the role of the meta trifluoromethyl substituent of the phenyl ring in inducing the silent agonist binding mode. Compounds MCP5, MCP6, MCP7, MCP8 were prepared by introducing halogen atoms, i.e. fluorine, chlorine, bromine, iodine with increasing size in the meta position of the phenyl ring; MCP18, instead, showed the original trifluoromethyl group moved on para position (Figure 1).

Two-electrode voltage clamping was employed to assess the electrophysiological profile of the newly synthesized compounds. Each experiment was conducted with 10 μM of drug, 60 μM ACh pre- and post-control, and an application of 10 μM of the type-II positive allosteric modulator, PNU-120596 to evaluate the induction of PAM sensitive desensitization. (5)

Taken together, our data suggest the meta trifluoromethyl group has a crucial role in minimizing the partial agonist behavior. Moreover, we found that the ability to stabilize the desensitized states of the α7 nAChR is preserved when trifluoromethyl is replaced by halogen atoms. When the CF3 group is moved on para position, the desensitizing activity is compromised, suggesting the meta substitution is strictly required.

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FRAGMENT BASED DESIGN OF O-GLCNAC TRANSFERASE INHIBITORS

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O-GlcNAc transferase (OGT) is an essential mammalian enzyme involved in the dynamic O-GlcNAcylation of cytosolic and nuclear proteins. Through catalyzing the attachment of N-acetylglucosamine to specific serines and threonines of proteins, OGT is associated with numerous biological processes such as transcription, the cell cycle progression, the stress response and nutrient sensing.1,2,3,4,5 In metabolic diseases like cancer and diabetes, increase of various metabolic products like glucose into the cell alters the production of UDP-GlcNAc through the hexosamine biosynthetic pathway. This promotes O-GlcNAcylation since OGT is highly sensitive to intracellular UDP-GlcNAc levels.6,7,8,9

To identify fragments targeting the donor UDP site, we have conducted a structure-based virtual screening in a fragment library containing more than 216,000 molecules. Among virtual hits, seven compounds contained the same scaffolds as they were all quinolone-4-carboxamides. A common feature of these molecules is that in the predicted binding mode the quinolone ring is anchored in the uridine binding site of OGT and the additional carboxamides point to the diphosphate binding site.

To further explore this finding, a series of 22 fragments carrying diverse carboxamides was prepared. The synthesis was conducted by coupling 2-hydroxyquinoline-4-carboxylic acid with various amines using EDC/HOBt to effect the coupling. The inhibitory potency of these compounds on OGT activity was measured using the UDP-Glo assay and several fragments were found to inhibit OGT activity. The most potent fragments were conjugated by short peptide with intent to reach improved synergy effect of the two component hybrid inhibitor.

References

Exogenously delivered messenger RNA (mRNA) shows a great promise as a therapeutic agent for gene therapies, cancer immunotherapies (mRNA vaccines) and stem cell medicine.\textsuperscript{[1]} mRNA possess several advantages over DNA in mentioned fields (e.g., mRNA does not need to integrate into the genome or be delivered into the nucleus to be expressed). Still, several limitations must be overcome before mRNAs can be considered as therapeutic agents. The 7-methylguanosine 5′ end cap structure is an ultimate hallmark of mRNAs. The cap structure can be subjected to extensive modifications, resulting in alterations to mRNA properties, and providing molecular tools to study mRNA metabolism.\textsuperscript{[2]} We present several dinucleotide cap analogs functionalized with azide groups, that were evaluated as reagents for the modification of mRNA 5′ ends.\textsuperscript{[3]} Using strain-promoted azide-alkyne cycloaddition (SPAAC),\textsuperscript{[4]} i.e. bioorthogonal, copper-free click chemistry, we were able to perform conjugation of modified mRNA with a Cy5 fluorescent dye derivative, both \textit{in vitro} and \textit{in cellulo}. Fluorescent labeling allowed for the cellular localization of exogenously delivered mRNA in HeLa cells. Importantly, the mRNAs before and after labeling procedure showed high translational activity both in cell extract and in living cells, which differentiates our approach from previously developed chemoenzymatic approaches to mRNA cap labeling and makes it of particular interest for translation-oriented research.\textsuperscript{[5]} The potential of our azide-functionalized cap analogs is not limited to fluorescent labeling; such modified RNAs could be conjugated with different alkyne-functionalized molecules, such as D-biotin, proteins, or nanomaterials, facilitating affinity purification, pull-down experiments, transport, and many others.

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DESIGN AND SYNTHESIS OF SMALL MOLECULES AS PROTEIN-PROTEIN INTERACTION STABILIZERS

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Targeting of protein-protein interactions has emerged as a significant strategy in drug discovery and despite the challenges there are successful examples.\textsuperscript{1} One class of proteins that participates in a number of PPIs is the 14-3-3 family of proteins. The interaction of 14-3-3 proteins with their binding targets leads either to inhibition or stabilization.\textsuperscript{2} More specifically, 14-3-3 proteins are positive regulators of p53, the tumor suppressor factor, which is mutated in approximately 50\% of human cancers. Therefore, one strategy is the stabilization of the PPI of 14-3-3 and p53, which is expected to lead to tumor suppression.\textsuperscript{3} In this project, we are focusing on the design and synthesis of small molecules with the potential to stabilize the PPI. Starting from a co-crystallized fragment, virtual libraries were designed and enumerated, taking into account multi-component reaction chemistry. Due to the fact that the binding surface was quite large, hydrophobic and without deep pockets, the designed scaffolds were flat and showed promising shape complementarity with the binding interface of the two protein partners. Regarding the synthesis, the scaffolds could be accessed via a two-step synthetic route. A library of derivatives, based on docking results, was synthesized. Currently screening and crystallization studies are on-going.

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References

LIPOPHILICITY AND METABOLIC STABILITY DETERMINATION ON NAPHTHO[1,2-d]/[2,1-d]OXAZOLES

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Lipophilicity and metabolic stability was determined on a series of naphtho[1,2-d/2,1-d] oxazoles. Compounds were synthesized by transformation of 4/5-styryloxazoles into naphtho[1,2-d/2,1-d] oxazoles by reaction of photochemical cyclization (1). Correlation was made based on structural properties of compounds. In order to determine the lipophilicity range of these compounds, Chromatographic Hidrophobicity Index was measured (2). It was determined that most compounds have favorable lipophilicity for targeting central nervous system diseases.

Metabolic stability was also measured and it was determined that most compounds did not have favorable stability. In the future consideration should be made on how to stabilize series of these compounds by making structural adjustments and determination of potential metabolic products will be made.

References
NOVEL POTENTIAL CANCER CELLS TRANSLATION INHIBITORS BASED ON PHOSPHORAMIDATE AND THIOPHOSPHORAMIDATE CAP ANALOG PRONUCLEOTIDES

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The cap structure is a modification present at the 5'-end of eukaryotic mRNA, which consists of 7-methylguanosine (m7Guo) linked to the first nucleoside of the transcript by 5'-5' triphosphate bridge. The structure has at least two major functions. It protects RNA from hydrolyze caused by exonuclease activity and initiates protein biosynthesis process through formation of the eIF4F translation initiation complex by interaction with eukaryotic Translation Initiation Factor 4E (eIF4E). It has been shown, that activation of the mTOR in the PI3K/AKT/mTOR pathway in cancer cells affects overexpression of eIF4E, which leads to dysregulation of the cap dependent translation. Moreover it has been also shown, that inhibition of expression of the eIF4E reduces tumor growth without toxicity. Therefore, the cap structure is an attractive starting point for the drug discovery. On the other hand, despite several cap analogues have demonstrated utility as therapeutics, their usability is limited by the low membrane permeability. To overcome this obstacle, there has been proposed use of pronucleotides for the in vivo delivery as one of the solutions to this problem.

We report novel pronucleotide 7-methylguanosine monophosphate (m7GMP) analogs containing phosphoramic or thio phosphoramic moiety obtained via chemical synthesis. Phosphoramic moiety was introduced through Yoshikawa phosphorylation followed by addition of ammonia to obtain new analog (1) or via Mukaiyama-Hashimoto activation and reaction with a tryptamine to obtain new analog (3). Another new analogs as two pairs of diastereomers (2a, 2b, 4a, 4b) containing phosphoramic moiety were obtained through modified Yoshikawa phosphorylation method followed by addition of a tryptamine or an ammonia. Each pair of diastereomers were separated using the RP-HPLC method. The phosphoramic moiety was expected to increase cell permeability owing to the phosphate charge masking effect whereas the thio phosphoramic modification was introduced to increase the affinity for eIF4E. We also performed a synthesis of previously reported translation inhibitor prodrug (5) as a reference for biological studies. Preliminary data on compounds stability in cell extracts will also be reported.

References
SUBSTITUTED 4,5’-BITHIAZOLES AS CATALYTIC INHIBITORS OF HUMAN DNA TOPOISOMERASE IIα

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The human DNA topoisomerase IIα (human topo IIα) is one of the major anticancer targets due to its role in the cell proliferative process. It catalyses topological changes of the DNA molecule, and plays an important role in biological processes, such as DNA replication, transcription and chromosome segregation, and its concentration is higher in rapidly dividing cells [1]. Because of its complex catalytic mechanism, several possibilities exist how to tackle this established anticancer target. Active agents targeting the human topo IIα are divided into two large groups; the established topoisomerase poisons [2] and an emerging group of catalytic inhibitors [3]. In our research we are using available structural information of the human topo IIα ATPase domain to rationally design new catalytic human topo IIα inhibitors that target the ATP-binding site on its ATPase domain. Such inhibitors prevent the native ATP ligand from binding, consequently stopping its catalytic cycle [2-4].

The starting point of this study comprised our discovered 4,5’-bithiazole compounds that were discovered to bind to the ATP binding site of the DNA Gyrase from E. Coli, the bacterial analogue of the human topo IIα [5]. By aligning the ATPase domains of the human topo IIα and that of the DNA Gyrase we determined the structural differences between their corresponding ATP binding sites. Based on these results a small focused library of 4,5’-bithiazoles was selected and screened against the human topo IIα ATP binding site. Analysis using obtained binding modes coupled with LigandScout-generated pharmacophores resulted in a selection of small series of compounds that were evaluated for its in vitro inhibitory activity. The best compounds showed activity in the lower micromolar range. In subsequent investigation we confirmed that these compounds do not act as topoisomerase poisons and further functional and biophysical assays suggested that they bind to the topo IIα ATPase domain. Compounds also displayed promising cytotoxicity and are a promising class for further development.

References
Infectious diseases remain one of the global health concerns due to increasing microbial resistance. Therefore, development of new antibacterial drugs with new mechanisms of action seems to be a promising concept of fighting against resistance. Bacterial topoisomerases represent one of the best established and validated targets in antibacterial drug discovery. DNA gyrase is type II topoisomerase and is composed of two GyrA (catalytic sites) and two GyrB (ATP-binding sites) subunits. Our research is focused on DNA gyrase B inhibitors and we have prepared a series of 4,5,6,7-tetrahydrobenzo[1,2-d]thiazole scaffold-based ATP-competitive inhibitors, which display enzyme inhibition in the low nanomolar range. However, penetration of bacterial cell wall and efflux still seems to be a major problem according to in vitro evaluation of antibacterial activity of these inhibitors. To solve the problem of penetration, a Trojan horse concept using siderophores was applied. Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms and are among the strongest known soluble Fe3+ binding agents. Bacteria exploit siderophores to transport iron into the cytoplasm and use it as an essential nutrient. This mechanism could be used to design novel DNA gyrase B inhibitor-siderophore conjugates to improve antibacterial activity of inhibitors.

Recently, we have designed and prepared new conjugates of DNA gyrase B inhibitors with hydroxypyranone- and pyocheline-based siderophore mimics. Compounds were screened for their inhibitory activity in the E. coli DNA gyrase supercoiling assay. The most potent inhibitor with hydroxypyranone moiety possessed activity in the low nanomolar range (IC50 = 90 nM, Figure 1). On the other hand, pyocheline-based siderophore conjugates showed weaker enzyme inhibition. We assume that pyochelin-like moiety is too bulky to fit the DNA gyrase B binding site. We examined also the effect of glycine linker on the activity for both series of compounds. Evaluation of antibacterial activity of conjugates in a low-iron conditions test system is in progress and will reveal the true potential of this Trojan horse concept, which will guide the future optimization.

**Figure 1:** A representative structure of siderophore conjugated DNA gyrase B inhibitor.

**References**

The ubiquitin proteasome system is a nonlysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle and apoptosis but also in inflammatory and immune processes, carcinogenesis, among other examples. Usually, in protein homeostasis the defective proteins are ubiquitinated and are proteolysed into short peptides by the proteasome. Proteasome substrates include, for example, signalling molecules, tumour suppressors, cell cycle regulators and transcription factors. Proteasome inhibition results in an interruption of the degradation of these substrates, leading to the activation of apoptotic pathways and, eventually, cell death. Rapidly growing cells, such as cancer cells, are particularly susceptible to proteasome inhibition mechanisms. [1][2]

This work relies on a computational-based drug discovery approach to find alternative new, selective (and more effective) small molecules as reversible proteasome inhibitors that can overcome the severe adverse drug reactions demonstrated by in use drugs. The efforts to discover new anticancer drugs described in this project combine different computer-aided drug design techniques (i.e. molecular docking, pharmacophore modeling, structure-based virtual screening, molecular descriptors calculation and molecular dynamics) in order to identify potential hit compounds (Figure 1). The selected compounds were tested in citotoxicity assays (i.e. MTT), being also performed inhibition assays for the chymotrypsin-like, trypsin-like and caspase-like activities of the proteasome using fluorogenic substrates.

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References
INVESTIGATION OF THE INTERACTION BETWEEN GLUCOCORTICOID RECEPTOR AND 14-3-3

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It has been estimated there may be as many as 650,000 protein-protein interactions (PPI) in human cells. Modulation of these interactions would potentially significantly enlarge the “drugable genome”. 14-3-3 is a family of seven highly conserved regulatory proteins and has been reported to interact with the glucocorticoid receptor (GR), a nuclear receptor which functions as a ligand dependent transcription factor, and modulate its activity. Different reports however have described both positive and negative regulatory roles to GR/14-3-3 interactions. Given the importance of GR agonists in medicine it is of great interest to better understand the role(s) of these interactions and to study their modulation.

In this work, the interaction between GR and 14-3-3 has been studied. Phosphopeptides, centered on putative 14-3-3 binding sites of GR, were synthesized and their affinity was measured with 14-3-3. Two peptides centred around T524 and S617 were the most active. A dimeric peptide based on these two joined by a pentaglycine linker was synthesized and determined to bind to 14-3-3 in the low nM range. The SAR picture for the importance of different residues to the binding was built up by an alanine scan. Finally these peptides have been crystallized with 14-3-3 (Figure 1).

Figure 1. Crystal structure of GR_T524-S617 and 14-3-3ζ

References
IN VITRO ACTIVITY OF 1-(DIPROPYLAMINO)-3-(AZACYCLOALKYL)PROPAN-2-YL (2-/3-ALKOXYPHENYL)CARBAMATES

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The emerging resistance of microbial pathogens to clinically used drugs, including second- and third-choice drugs, and development of cross-resistant or multidrug-resistant strains are alarming [1]. Thus, the antibacterial and antifungal potential of a series of dibasic 1-(dipropylamino)-3-(azacycloalkyl)propan-2-yl (2-/3-alkoxyphenyl)carbamates [2] was investigated in vitro. The R1 substituent was represented by a 2- or 3-alkoxy (from butoxy to heptyloxy) tail; a basic pyrrolidin-1-yl or azepan-1-yl moiety was chosen as a R2 substituent within a salt-forming fragment.

The highest in vitro antibacterial and antifungal activity was found for the compounds substituted by the pyrrolidin-1-yl ring. The activity was also determined by the length and position of the alkoxy side chain. The compounds containing a longer chain in C(3) of the phenyl ring were more active than their C(2) positional isomers. In addition, the most active compounds were tested for their synergy activity with clinical used antibiotics, and the dynamics of the activity was evaluated. No synergy effect against Staphylococcus aureus including a methicillin-resistant isolate and Enterococcus faecalis including a vancomycin-resistant strain was shown. The activity of currently investigated dibasic compounds was predominantly bacteriostatic. Based on the observed values of the surface tension of the most active molecules, their mechanism of action could be related to their surface activity.

References
SYNTHESIS AND EVALUATION OF NEW COLCHICINE DERIVATIVES AS POTENTIAL ANTIPROLIFERATIVE AGENTS

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Colchicine (1), a well-known tropolone alkaloid isolated from Colchicum autumnale, is of particular interest due to its antimitotic properties. It has played an important role in studies of mitosis and the therapeutic potential of colchicine binding site has been considered for chemotherapy applications [1,2]. However, colchicine itself as well as many of its derivatives could not be used as anticancer drugs because of their high toxicity. Up to now many structure-activity relationship studies have been done to elucidate the structural features required for the tubulin binding [3–5].

Herein, we report the synthesis, spectroscopic analysis of a series of novel colchicine derivatives, as well as evaluation of these derivatives as cytotoxic, tubulin-targeting agents.

The antiproliferative effect was tested in vitro on four human cancer cell lines and one normal murine embryonic fibroblast cell line (BALB/3T3). To better understand the interactions between the colchicine derivatives and tubulin, we also investigated potential binding modes of all studied compounds docked into colchicines binding site (CBS) of ß1 tubulin.

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\[ R = -\text{CH}_2\text{-O-CH}_3, -\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-Cl}, -\text{Ph}, -\text{CH}_2\text{-CH}_3, -\text{CH}(-\text{CH}_3)_2, (-\text{CH}_2)_3\text{-CH}_3, -\text{N}(-\text{CH}_2\text{-CH}_3)_2 \]

\[ X = \text{H, Cl, Br, I} \]
Flavin-dependent oxidoreductases monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) are important targets in the therapy of several neurological disorders such as depression, Parkinson’s disease and Alzheimer’s disease.\(^1,2\) As a part of our screening program devoted to discovery of new compounds targeting neurodegenerative diseases, styrilpiperidines were found to inhibit MAO-A and MAO-B. A comprehensive series of over 90 novel styrilpiperidines was therefore synthesized by applying systematic structural modifications on the benzene ring and by replacing piperidine with smaller or bigger saturated rings. 1,4-Disubstituted N-propargylstyrilpiperidines with trans-vinyl linker connecting piperidine and benzene ring irreversibly inhibit human (h)MAO-B with low nanomolar IC\(_{50}\) values. On the other hand, cis isomers irreversibly inhibit human (h)MAO-A with high selectivity over hMAO-B (Figure 1A). In contrast, derivatives with prolonged substituents (butinyl/pentinyl) on piperidine nitrogen displayed reversible inhibition of hMAO-B. To characterize the mechanism of MAO inactivation, co-crystallization and UV/visible spectroscopy experiments were performed. Crystal structures of four N-propargylstyrilpiperidines in complex with human MAO-B were resolved, thus confirming irreversible covalent adduct with FAD cofactor (Figure 1B).

Compounds are not cytotoxic to neuroblastoma SH-SY5Y cell line (EC\(_{50}\) > 100 μM) and display neuroprotective properties in cell based 6-hydroxydopamine model of Parkinson’s disease. They also display favorable in vitro pharmacokinetic parameters in terms of oral bioavailability and BBB permeability. Ex vivo experiments further demonstrate MAO-A and MAO-B inhibition after i.p. and oral administration in mice brain homogenates. Importantly, selective hMAO-A inhibitor 3 (Figure 1A) shows antidepressant activity in mice after i.p. administration (0.3 mg/kg) in chronic 10-day treatment regime.

**Figure 1:** Styrilpiperidines as selective MAO-A and MAO-B inhibitors.

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SMALL-MOLECULE IMMUNE CHECKPOINT INHIBITORS: NEW APPROACH IN CANCER IMMUNOTHERAPY

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Immunotherapy is currently a powerful strategy in cancer therapy with very exciting outcomes. In particular, modulation of immune checkpoint receptors have gain special attention. These immune regulators limit proliferation and activity of T cells and other immune cells enrolled in these signaling pathways. Under normal conditions, they are essential in modulation of immune responses; however, they are also one of the major mechanisms used by tumors to evade immune system recognition and destruction. To date, several immune checkpoint receptors have been identified and used as therapeutics in oncology, as programmed cell death protein 1 (PD-1). When engaged by one of its ligands (PD ligand 1 (PD-L1) and PD ligand 2) PD-1 limits autoimmunity. PD-1 ligands are upregulated in many human cancers and their blockade could lead to activation of T cells and therefore enforce tumor recognition. In fact, PD-1/PD-L1 pathway is one of the most successful pathways in the context of clinical cancer immunotherapy with several approved drugs. These successful therapies rely on the use of antibodies. However, despite their outstanding success, they still have numerous disadvantages as severe immune-related adverse events.

Recently, small-molecule modulators have emerged as safer therapeutic alternative. However, limited efforts have been directed toward immune checkpoint receptors. Our study is focus on the discovery of small-molecule inhibitors targeting PD-L1 in order to block PD-1/PD-L1 interaction and therefore overcome antibody therapy disadvantages. Limited structural information of PD-L1 led us to a detailed structural characterization based on in silico studies (molecular docking). After assessing structural features (e.g. flexibility and binding pocket) and following a computer assisted drug discovery approach we accomplished a structure based virtual screening campaign. Potential PD-L1 inhibitors were selected and their activity have been tested by Homogeneous Time Resolved Fluorescence (HTRF) assay. We were able to identify new small-molecule PD-L1 inhibitors that are currently being tested in vitro. Therefore, immune checkpoint blockade using small molecules represent a step forward in cancer immunotherapy.

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Glucose is regarded as the main fuel of cancer cells and the glycolytic pathway has been demonstrated as a potential target to be explored for cancer treatment. Several enzymes involved in glycolysis are overexpressed in different types of cancer cells, namely hexokinase 2 (HK2)\(^1\). This enzyme is involved in the first and most determinant step of the process, catalysing the phosphorylation of glucose to glucose-6-phosphate, also involved in the pentose phosphate pathway\(^2,3\). Therefore, the inhibition of the HK2 catalytic centre is proposed as a strategy to reduce the main source of energy to cancer cells, thus substantially decreasing cancer cell proliferation. As an effort to find hit compounds able to interfere with the HK2 catalytic centre and thereby block its activity, a structure-based drug design strategy was implemented, leading to the virtual screening of several general databases such as DrugBank (~2000 molecules), NCI (~265 000 molecules), Chemoteca (~800 molecules) and some specific natural products databases such as Inter Bio Screen Natural Products (~84 000 molecules), Human Metabolome Database Food (~40 000 molecules) and Enzyme Function Initiative - Phosphate sugars (~100 molecules). The virtual screening was carried out using molecular docking calculations through Gold 5.20 software. Molecules were prepared using Molecular Operating Environment (MOE2016 0802) and then docked into the HK2 catalytic site. Prior validation of the above-mentioned protocol was conducted, by testing different three-dimensional (crystallographic) HK2 structures, the amino acids at the catalytic pocket centre, scoring functions and catalytic pocket radius. Our results have suggested several hit compounds with the potential to act as new HK2 inhibitors that may progress to biological evaluation.

References
THE HIV-2 ENVELOPE GLYCOPROTEIN: STRUCTURE-FUNCTION RELATIONSHIPS TO STRUCTURAL ELUCIDATION AND CHARACTERIZATION

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The acquired immunodeficiency syndrome (AIDS) is the culmination of the infection by the human immunodeficiency viruses upon destruction of CD4+ lymphocytes of the host.1 The efficacy of the available antiretroviral drugs is very limited against HIV-2 and, most importantly, none of the current drugs effectively prevents entry into the cells. HIV envelope glycoproteins mediate binding to the receptor CD4 and co-receptors at the surface of the target cell, enabling fusion with the cell membrane and viral entry.2,3

The discovery of multiple new hit compounds that can be used as useful starting points towards drug candidates for HIV-1 and HIV-2 therapy is the main goal of this work. Until now, it has been marked by the use of computational techniques to study the viral surface glycoproteins as potential drug targets against HIV-1 and HIV-2 infections. The glycoproteins gp120 and gp125 are critical to the receptors recognition and internalization of viral material into the cell. The modulation of its activity can lead to the disturbance of this mechanism.

Our work has been focused in HIV-2 structure. In the absence of a crystallographic structure of HIV-2 envelope gp125 comprising variable domains, computer aided modulation is crucial to identify structural features in the variable regions that correlate with HIV-2 tropism and susceptibility to neutralization. HIV-2ROD is an X4 T-cell adapted isolate naturally resistant to antibody neutralization. A 3D structure of HIV-2ROD gp125 was generated by homology modelling, using MOE2016 and MODELLER 9v19. Additionally, to disclose the importance of the main structural features and compare with experimental results, 3D-models of six V3 mutants were also generated using the C2V3C3 domain. These mutations revealed selectively impact in the behaviour of the protein. Additionally, molecular dynamics is being performed, using Gromacs 2006, in order to better characterize this protein and disclose its the biological dynamic behaviour.

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DEVELOPING A RATIONALE FOR TARGETING THE RAS SUPERFAMILY

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Small GTPases are fundamental enzymatic switches, with roles in intracellular transport, receptor signalling, cell proliferation and development.1 Due to their vital role in the cell, dysregulation or mutations that affect their activity can result in disease, including cancer, autoimmunity diseases and dementia.2–5 Ever since Ras, a small GTPase was discovered as an oncogene, attempts have been made to produce small molecule inhibitors for these proteins, but efforts have been unsuccessful due to the underlying nature of these enzymes; the high picomolar affinity of GTPases for their nucleotide substrates has rendered competitive inhibitor strategies unproductive.6 During this PhD project, a novel approach to targeting the small GTPases will be made through the development of a competitive nucleotide-binding site inhibitor of a GTPase complexed to its effector, a guanine nucleotide exchange factor (GEF). It is hypothesised this will be more successful than previous attempts as binding of the GEF to the GTPase reduces the affinity of the GTPase for GDP/GTP from picomolar to micromolar.6,7 The propensity of the GTPase/GEF complex to form crystals, along with the necessity for structural information to ensure fragments are binding within the GDP/GTP binding site, makes it an ideal candidate for the XChem fragment screening technique developed by our group at the Diamond Synchrotron, UK.

This presentation will cover the early stages of this project, including high-throughput production of over 30 proteins to form GEF/GTPase complexes, the successful development of three crystal systems and fragment screening of 1000 compounds on Kalirin/Rac1 complex. Medicinal chemistry strategies using structure-based design for hits and expansion of the project to target neurodegenerative diseases will be discussed.

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DISCOVERY OF NEW ATP-COMPETITIVE HUMAN DNA TOPOISOMERASE INHIBITORS THROUGH BIOCHEMICAL SCREENING OF BACTERIAL DNA GYrase INHIBITORS LIBRARY

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Human DNA topoisomerase II plays vital roles in processes of transcription, replication and chromosome segregation and therefore represents an attractive target in anticancer drug discovery.1 It carries ATP-binding site that belongs to the GHKL superfamily together with bacterial DNA Gyrase, Hsp90, histidine kinase and MutL proteins.2 Based on the similarity of ATP-binding sites of human DNA topoisomerase II and bacterial topoisomerases, we screened an existing library of ATP-competitive bacterial DNA Gyrase inhibitors, that is a product of an extensive research work of our research group on discovery of new antibacterial agents,3–5 and used it as a starting point in discovery of new human DNA-topoisomerase inhibitors. This led to identification of a novel N-phenylpyrrolamide type of human DNA topoisomerase inhibitors. Further we used the newly discovered scaffold and structure-based design to synthetize optimized series of human DNA topoisomerase II inhibitors. The aim of optimization series preparation was to significantly decrease the molecular weights compared to original hits whilst maintaining the activity, giving new inhibitors an improved potential for hit-to-lead optimization. Cytotoxic activity of novel inhibitors was evaluated on MCF-7 and HepG2 cancer cell lines and one of the compounds showed activity comparable to one of clinically successful DNA-topoisomerase II inhibitor etoposide.

References
3D-PHARMACOPHORE-BASED APPROACH FOR DISCOVERY OF PROSPECTIVE EZH2 INHIBITORS

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Epigenetic pathways are recognized as determinants to cancer development and progression. Polycomb repressive complex 2 (PRC2) is an epigenetic regulator that catalyzes the trimethylation of lysine 27 in Histone 3 (H3K27me3), a process that facilitates chromatin compaction and gene silencing.1 The overexpression of EZH2, the catalytic subunit of PRC2, is implicated in the development and progression of a variety of cancers with the worst prognosis.2 Thus, EZH2 appears as a promising epigenetic target, and the development of new small-molecule inhibitors is currently a challenge.

We used computer-aided drug design (CADD) methods to identify new starting points for designing EZH2 inhibitors. Specifically, we created 3D-pharmacophore models, using LigandScout Advanced 4.2.1 software3 to support hit finding. In a first stage, a panel of unique pharmacophore models were generated. The performance of all models was validated against robust databases and the most predictive models were optimized further by systematic modification of the chemical features. The results revealed valuable information about the key interactions and the 3D-geometries associated with of EZH2 inhibition activity. The prioritized models were used for two hit finding campaigns: virtual screening and de novo design. First, using the unique 3D-pharmacophore-based virtual screening method (iscreen) from LigandScout, several databases (e.g., DrugBank, NCI, MuTaLig Chemotheca, and our in-house libraries) were computed and screened. Interesting hit molecules with high inhibition potential were found. Prioritized hits are being tested in biological assays to determine their EZH2 profiles. In parallel, we started a de novo design campaign based on selected pharmacophoric models. Those obtained from de novo design are being synthesized to further determine their EZH2 profiles.

Acknowledgements

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DIVERSIFYING THE PARKIN TOOLBOX - NEW CHEMICAL TOOLS TO FOLLOW PARKIN ACTIVATION

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Parkinson’s disease (PD) is the second most common progressive neurodegenerative disease, affecting approximately 1.5% of the population above 60 years old and 4% of the population at the age of 80 [1].

Although most of the PD cases are sporadic, it is now clear that genetic factors might contribute to the pathogenesis of the disease. For example, loss-of-function mutations in the parkin gene, which encodes Parkin protein, are the most common cause of autosomal recessive early-onset forms of PD [1].

Parkin is a ring-in-between-ring (RBR) E3 ubiquitin ligase, composed of six distinct domains. The catalytic module of PARKIN has a multidomain architecture comprising RING1, IBR and RING2 domains (the latter harbouring the catalytic cysteine), and is responsible for the ubiquitination and consecutive proteasome degradation of substrate proteins [2,3].

The ubiquitination-proteasome system is essential to numerous cellular events. Its malfunction induces impairment in mitophagy and accumulation of dysfunctional mitochondria, showing that loss-of-function of Parkin protein might be a key to the neurodegenerative process occurring in PD. Therefore, restoring Parkin function using rationally designed peptides and small molecules has been emerging as a potential therapy for Parkin-linked PD.

However, medicinal chemistry strategies to regulate Parkin pathway have always been constrained by the lack of suitable robust methodologies for screening endeavours [2,3].

To address this challenge, a series of activity-based probes for profiling Parkin activity is being developed. Concurrently, a yeast-based screening assay [4] is being implemented and the biological activity of selected probes evaluated.

These novel chemical tools hold promise as innovative biomarkers for Parkin activation, providing the bases for Parkin high-throughput screening campaigns.

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PROBING THE TORIN SCAFFOLD TO INDUCE PARASITE SELECTIVITY AGAINST THE MALARIA HEPATIC AND ERYTHROCYTIC STAGES

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Malaria, the mosquito-borne infectious disease caused by protozoan parasites of the Plasmodium genus, is an endemic disease in most tropical regions of the globe and still represents a major public health problem, with nearly half a million deaths reported just in 2016.[1] This infection progresses initially through a liver stage of parasite development, then followed by a blood stage cycle that is responsible for disease symptoms. Moreover, two species of Plasmodium, P. vivax and P. ovale, can remain latent in infected hepatic cells and be responsible for relapses and therapeutic failure.[2]

Despite the current therapeutic arsenal, parasite resistance is an established concern for most drug families and there are growing reports of increased tolerance to artemisinin in some parasite strains. These hurdles clearly demonstrate the necessity for development of drugs that display novel mechanisms of action that can overcome both the resistance cases and fill the existing void of liver stage active compounds. [3]

Torin2, an ATP-competitive mTOR kinase inhibitor, has been disclosed as a potent antimalarial with in vivo activity against both liver and blood stages. [4] Although no Plasmodium orthologs of mTOR exist, some proteins show high sequence similarity to the human mTOR at the kinase catalytic domain. This corroborates the hypothesis that Torin2 acts by a different mechanism of action compared to the drugs already in clinic, although due to its strong interaction with the human mTOR, Torin2 cannot be regarded as an ideal lead compound.

In order to reveal the structural features responsible for the antimalarial activity as well as those that relate to parasite-host selectivity, we built up a library of new Torin2 analogues, which were screened against both liver and blood stage parasites cultures, and we report the synthetic methodology as well as the structure-activity relationships (SAR) obtained in order to identify suitable lead compounds for further development (Figure 1). Equipped with that knowledge, and through minimally disruptive insertions of a photoreactive moiety and a handle for “click chemistry” we prepared a library of photoaffinity-based probes aimed at identifying the molecular target for this family of compounds.

Figure 1: Development of a chemically diverse library of Torin2 analogues, for the establishment of the SAR on Plasmodium spp. and the rationale for the development of the corresponding photo-affinity based probes.

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References
EVALUATION OF 4-THIAZOLIDINONE DERIVATIVES AS ALPHA-AMYLASE INHIBITORS

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Diabetes mellitus (DM) is a serious widespread chronic disease which is characterized by hyperglycaemia, due to reduced insulin production by pancreatic β cells and/or insulin resistance in peripheral tissues. Chronic hyperglycaemia is a major cause of metabolic imbalances and tissue damage which lead to blindness, kidney failure, heart attacks, stroke and lower limb amputation. Therefore therapeutic interventions in DM are mainly directed to control glycaemic levels and prevent diabetic complications.

Type 2 DM (DM2), which is the most common type of diabetes affecting more than 90% patients, is a multifactorial pathology that is generally treated with combinations of drugs directed at different biological targets. In recent years, it has been argued that a multi-target strategy, aimed at modulating simultaneously a number of selected targets with a single drug, can be a valuable alternative to multidrugs treatments [1].

Among enzymes involved in the regulation of glycaemia, α-amylase is a potentially attractive target for designing inhibitors able to control post-prandial glycaemia, which is a typical feature of DM2 and obesity [2]. This enzyme is secreted from salivary and pancreatic glands and catalyses the hydrolysis of a-1,4-glycosidic linkage in dietary carbohydrates, such as starch, producing maltooligomer chains. Other enzymes, like α-glucosidase, hydrolyse oligomaltose to glucose that can be adsorbed into bloodstream.

Currently, only few drugs active as α-amylase inhibitors are available, such as acarbose. This carbohydrate-derived drug also acts as α-glucosidase inhibitor and this could be responsible for unwanted effects. Therefore, the discovery of non-carbohydrate-derived inhibitors selective towards α-amylase over α-glucosidase appears to be desirable.

In the context of a research aimed at identifying multi-targeted inhibitors directed to enzymes involved in pathways related with DM, we evaluated a series of 4-thiazolidinone derivatives as α-amylase inhibitors. These compounds were selected from a panel of protein tyrosine phosphatase 1B or aldose reductase inhibitors, two enzymes implicated in the development of insulin resistance or diabetic complications. In particular, promising results were obtained with several aldose reductase inhibitors, which were found to be also able to inhibit α-amylase at low micromolar concentrations.

References

SYNTHESIS AND EVALUATION OF 7β-HYDROXY-8-KETONE OPIOID DERIVATIVES

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A large number of semisynthetic derivatives of opioid compounds have been developed over the years with many of them resulting from derivatization at positions 3, 6 and 17 of the opioid core. Modifications on the 7,8-double bond have been scarcely reported in the literature, and have mostly consisted of double bond reduction [1]. Oxidations of opioids are generally challenging and they have focused on the production of 14-hydroxy derivatives from thebaine and oripavine, in route to the preparation of drugs such as naloxone and naltrexone [2-3].

To study the effect of modifying the 7,8-double bond of opioids, we have developed a convenient, one-step, heterogeneous oxidation method for conversion of Δ^7,8-opioids into the corresponding 7β-hydroxy-8-ketones with potassium permanganate supported on iron(II) sulfate heptahydrate [4]. We have demonstrated that the oxidation reaction can be performed in the presence of various protecting groups, and studied the effect of the C6-substituent on the reaction outcome. 7β-Hydroxy-8-ketone opioids can be regarded as versatile intermediates for the synthesis of other opioids of interest.

The binding to and activation of opioid mu, delta and kappa receptors by the synthesized opioid hydroxy ketones was evaluated. The compounds acted as antagonists at the mu- and delta-receptors. Docking simulations and structure-activity analysis suggest that the newly introduced 7β-hydroxy-8-ketone functionality results in gain of activity towards the delta opioid receptor.

References

Covalent inhibitors play important role in drug discovery and therapeutics. About 30% of marketed drugs are covalent inhibitors, ranging from obesity to cancer.\(^1\) Although the toxicity of covalent inhibitors is a major concern, the advantages provided by them offer a large opportunity for further exploration. There are different warheads that act as covalent inhibitors, for example α,β-unsaturated carbonyl, epoxide, β-lactam, β-lactone, halomethyl, α-keto derivatives, etc.\(^2\) Multi-component reactions are powerful tool that can be used to synthesize covalent inhibitors. This work focused on synthesizing α,β-unsaturated carbonyl compounds, a Michael acceptor that binds covalently towards cysteine residue, through multi-component reactions.

References
Fluorescence methods are commonly known as one of the best way to monitor progress of enzymatic reactions or protein-ligand complex formation, due to their high sensitivity and selectivity. There are various fluorescence techniques that can be exploited for this objective, such as fluorescence intensity (FLINT), fluorescence polarization (FP), and fluorescence resonance energy transfer (FRET) measurements. The simplest approach is FLINT method, which does not require a complex experimental set-up. Furthermore, it can be used to monitor cleavage of small-molecule probes, in contrast to FP method which requires significant changes in size of the fluorescent molecule. However, in not every case fluorescence intensity changes are observed upon the cleavage. One of the solutions to this problem is using additional components, such as selective quenchers that can differentiate the fluorescence signals of cleaved and uncleaved probes. It has been reported that graphene oxide (GO), due to its large surface and the presence of the functional groups, can be used as selective quencher in enzymatic studies.\cite{1}

In our work, we employed the quenching properties of GO to study inhibition of Decapping Scavenger enzyme (DcpS), which is one of the proteins responsible for the degradation of mRNA 5’ cap structure. DcpS activity has been identified as a molecular target in Spinal Muscular Atrophy (SMA) and its inhibitors are potential therapeutics against SMA.\cite{2}

To obtain the fluorescent probe we performed CuAAC reaction between and azido-dye (6-FAM) and alkyne modified nucleotide (m7GTPanalog)\cite{4} or GTP and butynyl C-phosphonate as reference compounds. We characterized fluorescent properties of the synthesized analogs and studied their interactions with DcpS and GO. We observed that graphene oxide quenched the fluorescence of 6-FAM labelled m7GTP very strongly compared to non-methylated or butynyl C-phosphonate fluorescent analogs. Based on this phenomenon we developed a High Throughput Screening (HTS) assay for DcpS inhibition studies. To show the utility of our method, we applied it to characterize some of the known DcpS small-molecule inhibitors.

References

ANTICONVULSANT IDENTIFICATION AND QUANTIFICATION OF 1-(2,4-DICHLOROPHENYL)-2-(1H-IMIDAZOL-1-YL)ETHANONE O-BENZOYL OXIME

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Backgrounds: Epilepsy is a chronic neurological disorder characterized with spontaneous and concurrent seizures. Currently available antiepileptic drugs fail to control one third of the seizures, AKA therapy resistant epilepsies (TREs) and 6 Hz psychomotor test is used to identify potential agents to cure TREs. (Arylalkyl)azoles emerged as a new class of anticonvulsants with nafimidone and denzimol [1].

Aims: In this study, 1-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)ethanone O-benzoyl oxime (4) was synthesized and in vivo screened for its anticonvulsant activities in a two-step protocol by the Epilepsy Therapy Screening Program (ETSP) of the NIH. Its pharmacokinetic properties and possible anticonvulsant mechanisms were predicted in silico.

Methods: 4 was prepared by the reaction of 3 with benzoic acid in the presence of DCC and DMAP [2]. Its anticonvulsant identification was performed using 6 Hz and maximal electroshock (MES) tests in mice via ip route at two time points (0.5 and 2 h) and three doses (30, 100, and 300 mg/kg) according to the ETSP protocol [3]. Its neurotoxic effects were evaluated by minimal motor impairment tests. A number of physicochemical and pharmacokinetic properties and descriptors were calculated for 4 using QikProp; molecular docking studies were conducted using GABAAR homology model and extra precision Glide (Schrödinger, LLC, NY, 2018) [4].

Results and conclusions: In the identification test, 4 was found protective at 100 mg/kg in both models without toxicity. With ED50 and TD50 values in 6 Hz test at 0.5 h in mice via ip route was 118.9 and 241.7 mg/kg, respectively, 4 emerged as a promising lead for candidate molecules against TREs. 4 was found druglike and had favourable ADMET properties according to the Qikprop calculations. It showed high affinity binding to the benzodiazepine binding site of GABAAR model.

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References
Protein tunnels and gates are attractive targets for drug design [1]. Tunnels are important for the transport of ligands, solvent and ions, and can be found in many enzymes, ion channels and membrane proteins. To study the protein tunnels using a user-friendly graphical interface we have developed Caver Analyst 2.0 [2]. Caver Analyst can be used to identify tunnels in both static structures as well as molecular dynamics trajectories. Studying tunnels in protein assemblies from molecular dynamics simulations offers possibilities to observe transient tunnels and their changes in time. To study the transport of ligands through the protein tunnels, we have developed CaverDock [3,4]. CaverDock is fast, robust and accurate tool which allows the screening of binding and unbinding processes for pharmacologically interesting compounds. It is based on a modified AutoDock Vina algorithm [5] and we have previously successfully tested it with many pharmaceutically interesting targets, such as cytochrome P450 17A1 and leukotriene A4 hydrolase/aminopeptidase [6]. CaverDock is efficient method for virtual screening of compounds: one simulation took on average less than an hour and >90% of the studied cases led to a successfully calculated binding/unbinding trajectory. Caver Analyst 2.0 and CaverDock 1.0 are available free of charge at https://www.caver.cz/ and https://loschmidt.chemi.muni.cz/caverdock/.

References
ANTIMICROBIAL ACTIVITY OF SUBSTITUTED N-PHENYLHYDROXYNAPHTHALENECARBOXAMIDES

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Antimicrobial resistance is an increasing serious problem in the whole world. For this reason, the development of new active antimicrobial drugs is urgently needed. The series of N-phenylhydroxynaphthalencarboxamides were tested for their antibacterial activity against methicillin-resistant Staphylococcus aureus. MRSA was listed as the pathogen with high priority by the WHO. These strains are a frequent cause of hospital-acquired infections. In comparison with the methicillin-susceptible S. aureus, infections by MRSA have more than 50% higher mortality.

The activity of tested compounds was assessed by the microdilution method for determination of minimum inhibitory concentration. The antimicrobial potential was determined by the lipophilicity of compounds and the activity was also positively influenced by electron-withdrawing moieties. The most effective compounds were up to hundred times more active than reference ciprofloxacin.

In addition, the most active compounds were chosen for evaluation of dynamics of their effect by the time-kill curves method.

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3-FLAVONOLS AS NOVEL QUORUM SENSING INHIBITORS

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Bacterial biofilms are a major obstacle in the treatment of severe infections, especially cystic fibrosis and urinary tract infections.1 The low membrane permeability of antibiotics through bacterial biofilm results in enhanced resistance and ineffective treatment.2 Since bacterial cell-to-cell communication network, termed as quorum sensing (QS), plays a major role in the biofilm formation as well as pathogenicity and virulence, targeting QS will be more beneficial to tackle these bacteria.3,4 The goal of this Jane and Aatos Erkkos foundation-supported project is to develop novel QS inhibitors (QSI). Earlier, our colleagues have reported identification of 3-flavonols as low micromolar quorum-sensing inhibitors (QSI).5 Here in, we present our recent findings related to key structural features of 3-flavonols (Fig. 1, general structure I) required to maintain anti-quorum sensing activity. Our work show potential of 3-flavonols as potential QSI.

References
HETEROCYCLIC ANALOGUES OF SULFOCOUMARIN AS POTENT INHIBITORS OF CARBONIC ANHYDRASES

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Carbonic anhydrases (CA) are ubiquitous zinc containing enzymes. In humans α-CA catalyze a reversible hydration of carbon dioxide, and thus ensure regulation of intracellular pH and control transport of CO₂. There are 15 isoforms of α-CA. However, only two of them – CA IX and CA XII are overexpressed in hypoxic cancer cells, and they are involved in the control of optimal pH for survival and growth of tumor cells. Consequently, selective inhibition of CA IX and CA XII would result in blocking of cancer cells development without undesirable side effects. Good inhibitory activities of CA IX and CA XII were demonstrated of sulfocoumarin derivatives. [1, 2] Looking for a new selective CA IX and CA XII inhibitors we designed heterocyclic analogues of sulfocoumarin – thieno[2,3-e][1,2]oxathiine 2,2-dioxides and [1,2]oxathiino[6,5-b]pyridine 2,2-dioxides.

\[
\text{[1,2]oxathiino[5,5-b]pyridine 2,2-dioxide} \quad \leftrightarrow \quad \text{Sulfo coumarin} \quad \rightarrow \quad \text{Thienc[2,3-e][1,2]oxathiine 2,2-dioxide}
\]

The synthesis of sulfocoumarins and their heterocyclic analogues will be discussed.

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References
Membrane-bound pyrophosphatases (mPPases) can be found in many human pathogens including Plasmodium species, the protozoan parasite causing malaria. These large homodimeric integral membrane proteins generate an ion gradient across the acidocalcisomal membrane by hydrolysis of pyrophosphate (PPi). The mPPases are essential for the parasites as PPi is a by-product from many biosynthetic pathways and too high concentrations of PPi may disturb physiological reactions. Although mPPases can be found in many pathogenic protozoan parasites they do not exist in humans, thereby making them promising drug targets. The first structure of a mPPase was solved in the Goldman laboratory.

To date, only phosphorus-containing inhibitors of mPPases have been reported, limiting their therapeutic utility. Our aim is to develop novel protozoan mPPase inhibitors capable of disrupting the essential ion gradient of the pathogenic parasites in order to decrease their viability. Herein, we present a novel organic inhibitor of the Thermotoga maritima PPase through screening efforts. The compound inhibited the enzyme activity uncompetitively with an IC50 of 1.7 mM. In addition the binding mode was solved by X-ray crystallography at 3.7 Å resolution together with the substrate analogue, imidodiphosphate. The hit compound binds to the protein monomer near the exit channel, forming a hydrophobic clamp that lock the enzyme conformation in the closed state thus preventing hydrolysis and sodium pumping activity.

References
AZULENE-BASED COMPOUNDS TARGETING OREXIN RECEPTORS

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Orexin-A and orexin-B are the activating peptide ligands of G protein-coupled orexin receptors, OX1R and OX2R. The orexin signaling system has a central role in sleep-wake regulation. Therefore the orexin receptors could provide a clinical target for antagonism and agonism, to treat insomnia and narcolepsy, respectively. In recent years, the orexin receptor antagonists have been successfully developed, but the agonists have gained minor attention. Still most of the existing agonists are peptides, which are well known to be unsuitable therapeutic molecules and only one series of effective non-peptide orexin receptor agonists has been published to date.

In order to discover novel ligands for orexin receptors, we designed a virtual library consisting of 70,000 azulene-based compounds with substituents in the 1-, 3- and 6-position, which can be synthesized by our efficient synthetic methods for 1,3,6-trisubstituted azulenes. After docking the database to OX2R and visual examination of the top-scoring compounds, we selected a series of compounds for synthesis. With this approach, we identified novel orexin receptor ligands: both antagonists with Ki values in the low micromolar range and weak agonists. In addition, we discovered compounds that potentiated the orexin-A response to OX1 receptors two-fold at 10 μM.

References
LEAD OPTIMIZATION OF ISOXAZOLE DERIVATIVES TARGETING GATA4-NKX2-5 PROTEIN-PROTEIN INTERACTION RELEVANT FOR CARDIAC REMODELLING

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Ischemic heart disease leads to irreversible cell loss and is characterized by unmet medical need. Cardiac transcription factors, such as GATA4 and NKX2-5, regulate both physiological and pathophysiological processes in the heart. For example, a physical interaction of these two TFs leads to stretch-induced cardiomyocyte hypertrophy.1 In our previous studies we have demonstrated that inhibition of this protein-protein interaction (PPI) with a small molecule compound inhibits cardiomyocyte hypertrophy in vitro and improves cardiac function in vivo in experimental models of myocardial infarction and hypertension.2,3

In this study, we continued optimization of the original isoxazole hit compound by modifying its northern, central and southern parts. The new compounds were tested in the luciferase assay to examine the inhibition of the transcriptional synergy of the GATA4 and NKX2-5. Additionally, the most potent compounds were tested in luciferase assays for NKX2-5 and GATA4 separately. The generated three-dimensional activity data was analyzed by using hierarchical clustering to identify compounds capable of inhibiting PPI but not interfering with GATA4 or NKX2-5 DNA binding. Furthermore, toxicity of the compounds was studied with MTT and LDH assays in the COS-1 cell line.

In summary, we have synthesized and identified a group of non-toxic compounds, which inhibit transcriptional synergy of GATA4 and NKX2-5 without interfering with GATA4 transcriptional activity.

References
An analytical procedure by total attenuated reflection - Fourier transform infrared spectroscopy (ATR-FTIR) combined with chemometric instruments, was developed to detect adulteration of breast milk by addition of water or commercial cow milk. The FTIR spectra of 260 samples of breast milk, collected by the Milk Bank in the Hospital of Cosenza (Calabria, Italy), were recorded as pure samples and after piloted adulteration with whole, skimmed, partially skimmed cow milk and water. The experimental data were arranged in data matrices and handled by multivariate analysis. A first exploratory analysis was performed via principal component analysis (PCA), which allowed to extract information from FTIR spectral data and study both data and sample patterns. PCA clustering highlighted the presence of three distinct sample groups: pure breast milk and milk added with water or cow milk. In a second step, the algorithm partial least square - discriminant analysis (PLS-DA) was used to build a classification model able to distinguish with high reliability the pure breast milk and the four groups of adulterated samples and also to estimate the cause of the adulteration. The classification model was validated on external samples that reached 100% of the correct prediction for pure breast milk, some difficulties were found in the PLS-DA prediction about the type of cow milk added to breast milk, where the correct classification was 90%. In order to optimize the quantification of added water or cow milk, four PLS calibration models, specific for the different adulterants, were built. External validation of these models provided satisfactory statistical parameters with low root mean square error (RMSEP) and relative error (RE%) below 1.38 and 3.31, respectively.
SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF CB2 HOMOBIVALET LIGANDS

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G-protein-coupled cannabinoid receptors, CB1 and CB2, have emerged as promising therapeutic targets with a high potential for the treatment of cancer, pain or neurological disorders among others. As other GPCRs, CB1 and CB2 present a rather complex molecular pharmacology. The existence of distinct binding sites, different effector-coupling proteins, biased modulation, or oligomerization processes govern their intricate functionality. In this context, bivalent ligands may allow the study of multifunctional receptor activity and can provide receptor type-selectivity.

Few bivalent ligands have been described for the cannabinoid receptors; most of them target the CB1 receptor. Heterobivalent ligands targeting CB1 and opioid receptors have previously been developed by us.1 Herein we report the identification of CB2 selective bivalent ligands based on the chromenopyrazole scaffold previously described by us as cannabinoid ligand.2

A series of homobivalent chromenopyrazoles containing alkyl chains as spacers and their respective univalent 9-alkoxychromenopyrazole analogs have been synthesized. Their ability to bind to cannabinoid receptors was measured through radioligand assays observing full selectivity towards the CB2 type eliminating the psychotropic effects related to the CB1 type. Functional cAMP assays performed in HEK293 cells overexpressing recombinant human CB2 receptors showed their CB2 agonist profile. Interestingly, their univalent analogs were not able to orthosterically displace[^CP55940^] in radioligand binding assays. However, functional studies are currently ongoing to assess their potential allosterism. To further investigate if the bivalent ligands act as dualistic/bitopic CB2 agonists modeling and mutational studies are being undertaken.

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References
NMR-ASSISTED DETERMINATION OF SPECIFIC BINDING OF STILBENE DERIVATIVE TO BACTERIAL MUR LIGASE D

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Mur ligases (MurC–F) are ATP-dependent intracellular bacterial enzyme, essential for the biosynthesis of the bacterial peptidoglycan. They represent attractive targets for the development of novel antibacterial agents. The intracellular steps of peptidoglycan synthesis have been greatly underestimated and only two such antibacterial agents are in clinical use by now. We have tested binding of a library of kinase inhibitors against Mur ligases (MurC–F), which could have potential for development of multitarget inhibitors of Mur ligases. The most potent one, a stilbene derivative, was selected for NMR studies to determine its specific binding to the 47.7 kDa Mur ligase D (MurD). We have already determined the specific binding of several MurD inhibitors with various molecular scaffolds using the extensive NMR studies. We were able to identify in NMR spectra the crucial MurD signals that are influenced at ligand binding either to ATP or uracil or D-Glu binding sites.

First we applied the STD NMR method with a large excess of stilbene derivative to confirm its binding to MurD. Before proceeding to the protein-based NMR studies, we tested the effect of different additives and ligands on MurD stability, using Differential Scanning Fluorimetry (DSF). The tests were performed on unlabelled MurD with increased DMSO concentrations as an additive and a set of ligands: AMP-PCP, a sulfonamide inhibitor and a stilbene derivative. Results show that the presence of DMSO in a concentration range from 10% to 14% has no significant effect on MurD stability. The ligands were therefore solubilised in DMSO and their effect tested. Among the ligands tested, stilbene derivative showed no effect on stabilisation, whereas AMP-PCP and sulfonamide inhibitor displayed a 1-2 °C stabilization on MurD.

The specific binding of stilbene derivative was investigated using the NMR HSQC-based titration experiments. We prepared isotopic labeled Escherichia coli MurD protein. MurD was heterologously expressed in E. coli strain BL21 (DE3). We optimised the expression and isolation protocol in order to increase yield and purity of the labeled protein. For the preparation of 2H/15N labeled and selectively 13C labeled MurD, the cells were gradually adapted to increased D2O concentration in M9 media and finally grown and induced in 2H/15N M9 media with labeled α-ketobutyrate and α-ketoisovalerate. The map of chemical shift perturbations of selectively labelled methyl groups of MurD upon binding of stilbene derivative clearly indicate its binding to D-Glu binding site located in the enzyme C-terminal domain. While the methyl groups of the enzyme central domain, which are largely perturbed upon binding of AMP-PCP, remain unaffected. The determination of the binding site of selected stilbene derivative offers the basis for the development of more potent stilbene type multitarget inhibitors of Mur ligases.

This work was supported by the Slovenian Research Agency (Grant numbers J1-8145, P1-0010 and P1-0208) and EN-FIST Centre of Excellence.
PHYTOCHEMICAL ANALYSIS OF AN AUSTRALIAN NATIVE PLANT AGAINST COMMON WOUND-COLONISING BACTERIA

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Background

The global emergence and spread of multidrug-resistant bacteria has limited the efficacy of current therapeutics resulting in treatment failure and infection recurrence (1). Recently, antibiotic resistant bacteria have escalated, as resistance to different drug classes such as penicillins, cephalosporins and sulphonamides have continued to increase (2). Plants are able to produce different types of biologically active compounds, many of which have reported antibacterial effects (3). The increasing interest in traditional medicine provides an alternative form of therapeutic for the treatment of various microbial infections.

Methodology

Ground dried leaves of an Australian native plant (denoted species 8484) were extracted with different solvents. The extracts were screened against nineteen wound-colonising bacteria using the well diffusion assay. Each assay was performed in triplicate and final values were expressed as mean values ± SEM. The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the effects of plant extracts on the formation of monomicrobial and polymicrobial biofilms were also determined. Further, extracted compounds were separated by reverse phase high performance liquid chromatography (HPLC) which were then identified and evaluated by nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Results

The extracts obtained from the selected plant (100 mg/mL) showed an antimicrobial activity against nineteen wound-colonising bacteria. Crude aqueous (100 mg/mL) extract showed higher levels of antimicrobial activity compared with extracts obtained using other solvents. The methanolic extract of selected plant species had a MIC of 2µg/mL against E. faecalis. In contrast, a MBC of 20 mg/mL from the methanolic extract was shown to exert the greatest bactericidal effect against S. pyogenes. Results of both the monomicrobial and polymicrobial biofilms showed that with increasing concentrations (0, 20 30 and 40 mg/mL) of the methanolic plant extract, the formation of monomicrobial (E.coli) and polymicrobial (MRSA and P. aeruginosa) biofilms decreased. The isolation and identification of bioactive compounds using HPLC and NMR have identified four flavonoids i.e. (+)-catechin, (+)-taxifolin, (+)-aromadendrin and farrerol.

References

DENSITY AND PARTIAL MOLAR VOLUME OF N,N-DIMETHYL-N-[2-[(1S,5R)-8,8-DIMETHYL-2,4-DIOXO-3-AZABICYCLO-[3.2.1]OCTAN-3-YL]ETHYL]OCTADECANAMMONIUM BROMIDE IN THE PRESENCE AND ABSENCE OF KCl, NaCl, NaBr AND KBr IN AQUEOUS SOLUTIONS

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The critical micellar concentration of studied tenside N,N-dimethyl-N-[2-[(1S,5R)-8,8-dimethyl-2,4-dioxo-3-azabicyclo-[3.2.1]octan-3-yl]ethyl]octadecanammonium bromide in aqueous solution and in the presence of NaCl, KCl, NaBr and KBr was determined by pycnometric density measurements at laboratory temperature (296.15 K). Concentrations of salts varied in interspace from 0.01 to 0.1 mol/dm³.

On the basis of our results of density measurements were also determined partial molar volume and degree of dissociation in distilled water and in presence of inorganic salts NaCl, KCl, NaBr and KBr.

Our experimental results show, that it is assumed that the individual inorganic additives differ in the value of critical micelle concentration, because the increasing concentration of chloride anions Cl⁻ decreases the CMC value, so it is easier to form micelles. Further evidence of the different influence of the additives is, that with increasing concentration of bromide anions Br⁻, the CMC rises. Individual salt cations (Na⁺ and K⁺) also affect CMC.

Furthermore, we have observed that the value of the partial molar volume is higher in aqueous solution than in solutions with the addition of inorganic salts. The partial molar volume is lower in the presence of KCl than in the presence of NaCl. Analogous results were observed in the presence of bromides, the partial molar volume decreasing in the order of aqueous solution, NaBr solution, and KBr solution. Degree of dissociation was calculated for all solutions.

Keywords: Critical micelle concentration. Micellization. Pycnometry. Partial molar volume. Degree of dissociation.
Type 2 diabetes mellitus (T2DM) is a common disease affecting 9% of the world population in 2014. Number of people suffering from T2DM is expected to exceed half a billion by 2030. One of the promising targets for treatment of these patients is the liver glycogen phosphorylase (lGP) which is the key enzyme of liver glucose production. Since GP catalyses a reversible reaction, activity measurement methods based on glucose-1-phosphate (G-1-P) production or the reverse glycogen synthesis have been developed. There are numerous known GP inhibitors, but the inhibition data of literature are not always comparable. Inhibition data, obtained from measurements using synthesis (mainly inorganic phosphate (Pi) by spectrophotometry) and breakdown (mainly coupled enzyme assay) reaction conditions, are often different. We hypothesized that measured values differ due to the different reaction conditions of the measurements.

Our aim was to develop an ITC method which is suitable for activity measurement of GP enzyme in both direction of the catalysed reversible reaction. The substrate specificity of rabbit muscle glycogen phosphorylase b (rmGPb) was studied on substrates of different length. CNP-G7 was selected as a substrate for the optimization of reaction conditions since due to the CNP chromophore group its reaction can be monitored by HPLC, as a control method. The second substrate determines the direction of the reaction: in presence of G-1-P the synthesis, while in presence of inorganic phosphate the phosphorolytic reaction occurs. Varying the G-1-P/Pi ratio systematically, we could achieve the appropriate dominance of synthesis or degradation at 10 and 0.1 ratio, respectively. Same ratios were applied for inhibition studies on glycogen substrate applying some known GP inhibitors (glucose, caffeine and glucopyranosylidene-spiro-thiohydantoin (GTH)) on both directions.

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Cystatin C (hCC) is a small globular protein produced by all nucleated cells of the human body. Its primary function is the inhibition of cysteine proteases, but it also has neuroprotective and antioxidant properties. A naturally occurring mutation (L68Q) in the hCC gene results in a severe neurodegenerative disease called hereditary cystatin C amyloid angiopathy. The mutant oligomerizes easily and forms aggregates which are deposited in the blood vessels of the brain. This process leads to strokes, intracerebral haemorrhages and finally young adults death. Due to hCC functions in the body and its amyloidogenic properties, it constitutes interesting research subject.

Many studies on amyloidogenic proteins such as amyloid β and α-synuclein indicate the important role of metal ions (e.g. copper, iron and zinc) in their oligomerization process. In our research, we have studied the influence of copper on the cystatin C and its variants aggregation. Proteins were incubated with Cu(II) ions at 37 °C for up to six days. Samples were analysed using size-exclusion chromatography (SEC). Results indicate a significant acceleration of the cystatin C oligomerisation connected with oligomers precipitation. This effect may also apply to other transition metals and affect the hCC aggregation in vivo.

Thermal induction of the cystatin C expression, even though efficient, fails in the case of less conformationally stable variants (L68Q and analogues). Therefore we have constructed new, pET-based vector for temperature-independent expression of hCC and its mutants. Efficient gene cloning, high-level protein expression and searching for factors affecting the oligomerisation process are necessary for proper understanding of cystatin C and other amyloidogenic proteins pathogenicity.

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DEHYDROABIEATIC ACID DERIVATIVES TARGET BACTERIAL BIOFILMS

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Bacterial biofilms represent a major threat due to their remarkable resistance to conventional antibiotics and involvement in hospital-acquired infections (HAIs).1 For example, hospital-acquired pneumonia has been related to a mortality rate of 70% and above, either directly or by contributing to other factors. In addition, other HAIs lead to 7-9 additional days of hospitalization which causes a worldwide financial burden.

Given the facts, it is necessary to synthesize new effective anti-biofilm agents that can inhibit biofilm formation and/or kill established biofilms. Recently, our group discovered a new class of hybrid compounds using dehydroabietic acid, a diterpenoid from coniferous trees, as a starting material. Two of the designed compounds are the most potent abietane-type anti-biofilm agents reported so far in literature (Figure. 1), targeting staphylococci including Staphylococcus aureus.2

The results discovered showed that diterpenoids from coniferous trees represent an excellent starting material for anti-biofilm agents. The ongoing research in our lab focuses on exploring and optimizing more diterpenoid derivatives to target bacterial biofilms. Standard structural elucidation techniques are used to confirm the structure of the synthesized compounds.

References
RATIONAL DESIGN, SYNTHESIS AND RADIOLABELLING OF NOVEL POTENTIAL CCK₂R ANTAGONISTS

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Aim. The CCK₂/gastrin receptor (CCK₂R) is overexpressed on several tumours, and is therefore a suitable target for the development of the radiolabelled binding ligands, which are used in nuclear medicine for SPECT, PET, and/or PRRT. The radiolabelled CCK₂R agonists, exhibiting some pentagastrin test-like adverse effects, are being currently evaluated. Considering the recently proven fact for the SSTR/somatostatin system that the internalization is not required to obtain high tumour uptake and sufficient retention1, we suggest the same switch from agonists to antagonists also for the CCK₂R system. The aim of the present study was therefore to design, synthesize, and radiolabel the potential novel CCK₂R antagonists.

Methods. In silico structure-based drug design was used for the design of novel CCK₂R antagonists. The homology model of CCK₂R was built based on the X-ray structure of β₂ adrenergic receptor (PDB code: 2RH1). Docking of energy minimized structures of the molecules, comprised of known antagonist Z-360 and different spacer, was performed using GOLD 5.5. The best scored potential DOTA-conjugated antagonists were further modified by the addition of hydrophilic linker to obtain soluble compounds. The synthesis was carried out on solid phase with standard Fmoc method. The products were purified using semi-prep HPLC and evaluated with ESI-MS and HRMS. The purified compounds were radiolabelled with ⁶⁸Ga, ¹¹¹In and ¹⁷⁷Lu, and the yield of radiolabelling was determined using radioHPLC. The logD₇.₄ values were determined for radiolabelled compounds using shake-flask method.

Results. Based on the values of GoldScore scoring function and visual inspection of docked structures, we have determined the minimal number of amino acids that are necessary for the unhindered binding of DOTA-conjugated molecules to exit the binding pocket. The potential DOTA-conjugated antagonists that showed additional interactions in the spacer region were selected for synthesis. All synthesized conjugates showed purities over 95% as confirmed by reversed-phase HPLC. Molecular weights were confirmed by ESI-MS and HRMS. The optimization of the conditions of radiolabelling resulted in radiolabelling yields over 90%. The logD₇.₄ values were between -2.7 and -2.5 for all compounds.

Conclusion. Several novel potential CCK₂R antagonists were designed, synthesized, and radiolabelled with different radionuclides, and are therefore suitable for the further in vitro evaluation of binding and agonistic/antagonistic properties.

References
Two novel conjugates of pyrene and cyanine were constructed by linking them with a rigid triazole–peptide linker. These new probes bind very strongly (with 0.1 mM affinity) to both ds-DNA(RNA) and proteins (BSA), giving significantly different fluorimetric responses: a strong pyrene emission change is highly selective for proteins and the “switch-on” of cyanine fluorescence is highly selective for DNA(RNA). Moreover, the new probes yield induced CD bands only with DNA/RNA, but not with BSA, which allowed an independent check of DNA presence in DNA/protein mixtures. Furthermore, these probes contain a FRET pair of chromophores, whereby FRET is silent in a free molecule solution and is activated by binding of the small molecule to the biomacromolecular target. The efficiency of FRET is to some extent related to the secondary structure of DNA/RNA and only for one of the probes is FRET activated in proteins. The two probes show distinctively different induced CD patterns in the 400–600 nm range (attributed to a different position of linker attachment on the cyanine core), allowing differentiation between various secondary structures of DNA or RNA, which are shown to be additionally enhanced by combining pyrene and cyanine into one molecule. Due to their low cytotoxicity and efficient cellular uptake, these probes are good candidates for further biological studies.

References
STUDY OF THE OLIGOMERIZATION MECHANISM OF THE HUMAN SAA1.1

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Aggregates of the human serum amyloid A (hSAA) protein are dangerous for parenchymal tissues.1 Our aim is the elucidation of the mechanism of formation of these harmful structures. We have performed our studies using not only the full length hSAA but also the simpler models, derived from the sequence of the most amyloidogenic hSAA1.1.

Human SAA is a small protein consisting of 104 amino acid residues. The monomer is composed of 4 α-helices, which are oriented anti-parallely to each other, forming a cone shaped structure. In physiological conditions hSAA exists as an oligomer, most probably a hexamer.2 Its role is not fully understood, but it is known that it participates in the transport and metabolism of cholesterol. Intensive production of SAA occurs during chronic inflammation, accompanying, for instance, rheumatoid arthritis.3 A prolonged accumulation of SAA molecules can cause their oligomerization and aggregation. Amyloid fibrils of hSAA are constructed mainly from the 1-75/1-76 fragments.4 Two putative scenarios of their formation can be taken into consideration: 1. cutting off the C-terminus, which stabilizes the protein structure, causes its unfolding and subsequent creation of pathological aggregates and fibrils, 2. formation of fibrillar structures exposes the C-termini and enables their cutting off.

We have performed our research starting from the peptidic models derived from the N-terminal sequence of the protein. One fragment consisted of 27 amino acid residues and showed similar helical structure and fibrillization properties to the whole protein. Interestingly, the larger protein fragment (hSAA1-75) did not tend to be organized.

In our research we used circular dichroism, fluorescence and size exclusion chromatography methods.

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References
MOLECULAR MODELING OF S. AUREUS FLAVOHEMOGLOBIN TO PROVIDE INSIGHTS INTO ANTIBACTERIAL EFFECTS OF SOME AZOLES

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Backgrounds: Azole antifungals such as miconazole and econazole are known to have potent antibacterial effects through inhibition of bacterial flavohemoglobin, an enzyme catalyzes the conversion of NO to NO₃⁻ thus relieves nitrosative stress. Inhibition of bacterial flavohemoglobins by azoles have recently been described through X ray crystallography studies [1].

Aims: In this study, we built a homology model for Staphylococcus aureus flavohemoglobin (SAFH) and performed docking studies of an in house azole library that showed potent antibacterial effects against S. aureus to validate SAFH inhibition possibly underlying this effect.

Methods: Homology model of SAFH was created using the crystal structure of R. eutrophus flavohemoglobin (PDB ID: 3OZU [1]) as template according to comparative modelling techniques on MODELLER [2]. Structural quality of the model was analysed by Procheck [3]. The model was then prepared for docking using Protein Preparation wizard of Maestro (2018-1, Schrödinger, LLC, New York, NY, 2018). The ligands were prepared and minimized using MacroModel (2018-1, Schrödinger, LLC, New York, NY, 2018) with OPLS 2005 force field parameters. Docking studies were performed using extra precision Glide [4].

Results and conclusions: SAFH was successfully modelled according to the Procheck analysis. Docking studies showed that the azole ring of the compounds form the axial 6th axial coordination with the iron of the heme co-factor present in the catalytic site of SAFH. Other moieties were mostly in hydrophobic contacts with the active site residues.

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References
INSIGHTS INTO THE NERVE GROWTH FACTOR / ENDOGENOUS LIGANDS BINDING MECHANISM

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Nerve Growth Factor (NGF) is the prototype of the neurotrophins family and induces cell growth and differentiation in neuronal cell types, both in the central and peripheral nervous system. Even though it was discovered almost 70 years ago, and its tertiary structure is known since 1991, many molecular and functional properties remain elusive. At the same time, its pharmaceutical interest is still high, since the protein is involved in many disease mechanisms, like chronic pain and neurodegenerative disorders.

Small endogenous ligands that interact with NGF are of increasing interest, due to their likely capability of modulating its biological activity. Among these molecules, ATP was shown to mediate NGF neurotrophic activity through its receptors, TrkA and p75NTR. However, no structural information on the binding sites nor on the mechanism of the interaction is available so far. Aiming at gaining new information on this aspect, we undertook a biophysical study on NGF/ATP binding, by means of solution NMR.

We have focused our studies on the recombinant human NGF (rhNGF), which is the molecule of medical interest, more than the better structurally characterized mouse protein (mNGF). At first, we have obtained $^{15}$N- and $^{13}$C$^{15}$N-labeled rhNGF, suitable for the NMR studies. We have optimized the protocols set up in our previous work on the mouse protein, to better adapt the expression conditions to the human protein. The 2D HSQC NMR collected spectra allowed us to identify structural features of the rhNGF in comparison to mNGF in solution. We know from our previously published data that the hNGF and mNGF do not overlap in their biochemical, biophysical and in vitro functional properties, reflected in their 3D structure in solution and highlighted by their available respective crystal structures. We therefore proceeded to the assignment of both the backbone and side chains of hNGF, to fully characterize the protein, by means of 3D NMR experiments ($^{15}$N and $^{13}$C NOESYs).

We investigated the binding effects of ATP and of a set of different divalent ions by means of Differential Scanning Fluorimetry and identified the suitable conditions for the NMR studies. We then moved to the investigation of the NGF/ATP binding, using a protein-based solution NMR approach. We recorded 2D HSQC spectra following a titration with increasing amounts of ATP or the more stable analogue, ATP-PCP. We could identify the binding site of ATP and the results will be described.

The work was supported by the Slovenian Research Agency (Grant numbers J1-8145 and P1-0010).
The Epidermal Growth Factor Receptor (EGFR) is a key target in anticancer research, whose erratic function in malignancies has been associated with severe irregularities in critical cellular processes, including cell cycle progression, proliferation, differentiation and survival. EGFR mutants, either transmembrane or translocated to the nucleus and/or the mitochondria, often exhibit resistance to EGFR inhibitors.

With a broader scope of developing small-molecule inhibitors able to target mutant forms of EGFR-TK and simultaneously monitoring their intracellular distribution, we have developed a synthetic approach that combines an aminooquinazoline, found to inhibit EGFR-TK, with a ruthenium(II)/bipyridine-based fluorophore, via a versatile triethylene-glycol-derived spacer. The resulting conjugate exhibits an unprecedented 2:1 ratio of inhibitor units relative to fluorophore units due to the connecting ability of the spacer.

In U87MG (grade IV malignant glioma) cells, the conjugate is effectively uptaken and shown by fluorescence imaging to exhibit specific sub-cellular distribution that coincides with the distribution of a mitochondria-targeting dye. A time-resolved Förster resonance energy transfer study (TR-FRET) and mitochondrial isolation assay are consistent with mitochondrial localization. The property of the conjugate to accumulate in mitochondria is attributed to the positive charge from the complex and the ionizable positions of the spacer, and could be valuable in developing next-generation inhibitors that target mitochondria-residing forms of EGFR-TK.
SYNTHESIS OF AMINOCHALCONES AND EVALUATION OF THEIR ANTIMICROBIAL AND ANTICANCER ACTIVITY

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Chalcones belong to a large group of polyphenolic compounds known as flavonoids. They are widespread in plant kingdom and play an important function as secondary metabolites which protect from damage caused by microorganisms, insects and animals. Moreover, they exhibit a variety of biological activities such as antioxidant, anticancer, anti-inflammatory, antibacterial, antifungal, and antimalarial [1]. Biological potential of chalcones is the result of their chemical structure based on an α,β-unsaturated linker between two aromatic rings. In view of those properties, they still have an unflagging interest among scientific community.

The aim of our research was to synthesize a library of aminochalcones in classical Claisen-Schmidt reaction [2]. In aldol condensation of 2'-amino-, 3'-amino- and 4'-aminoacetophenones with variety of benzaldehyde derivatives, 18 aminochalcones were obtained with yield up to 88.6%. Among all synthesized compounds, 10 derivatives were novel, have never been described before in scientific literature. Structures of all products were confirmed by 1H and 13C nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS).

To verify biological properties of obtained compounds, antimicrobial and anticancer activity were evaluated. The antimicrobial activity was tested on two strains of bacteria: Escherichia coli and Staphylococcus aureus, and four strains of fungi: Candida albicans, Alternaria alternata, Fusarium linii and Aspergillus niger. To prepare the growth curves of each microorganism in presence of aminochalcones, optical density at regular intervals was measured. As a result of this investigation, complete inhibition of E.coli growth was observed in presence of almost all derivatives. The anticancer activity assay was performed on four different types of human colon cancer cell lines (HT-29, LoVo, LoVo/dx and LS180). The cytotoxicity was calculated as IC50 value and compared to reference compounds – doxorubicin and cisplatin. Majority of tested compounds showed IC50 values below 5 µg·mL-1.

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References
Drugging the FBW7 E3 Ligase with a Fragment-Based Approach

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FBW7 is an important E3 ligase and one of the most commonly deregulated proteins in human cancers. 6% of cancers have mutations in the FBW7 gene. In one hand, the loss of activity of the mutated FBW7 results in a loss of its tumour suppressor function and an upregulation of the natural and oncogenic substrate proteins, such as c-Myc, cyclin-E, and Notch.1 On the other hand, the inhibition of FBW7 has been proposed as an approach to sensitize cancer stem cells to chemotherapies.2 Given the key role of FBW7 in tumorigenesis, a small molecule directly targeting FBW7 would have a large impact on the clinic. However, so far, no potent small-molecules that directly bind to FBW7 have been reported, in part because modulating their activity and regulation requires targeting protein-protein interactions.3

Our goal is to identify and characterize fragments that bind to the FBW7 E3 ligase and can be further developed as chemical probes. These fragments may turn on or off the activity of the protein. FBW7 binders could serve as anchors to develop disease-specific PROTAC molecules, leading to proximity-induced ubiquitylation and subsequent degradation of proteins of interest.4 Our group has built a library of around 700 fragments. Surface Plasmon Resonance (SPR) has been carried out. Potential fragment-hits have been identified and they are being validated using orthogonal biophysical techniques. Furthermore, in order to elucidate the binding mode of the fragments, it is crucial to perform x-ray crystallography. Crystal structure of fragments binding to the protein will not only show the key points for the interaction but also it can provide the starting point for a rational design to grow the molecules in order to improve their affinity and specificity.

References
SYNTHESIS OF NOVEL PYRAZOLO[1,5-a]PYRIMIDINE BASED CATHEPSIN K INHIBITORS

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Cathepsin K is a cysteine protease, which can degrade type I collagen. It is expressed in osteoclasts and plays an important role in bone resorption. It is associated with degenerative bone diseases, including osteoporosis1. By inhibiting cathepsin K selective reduction of bone resorption would be possible. Many currently used osteoporosis treatments include estrogen replacement therapy and bisphosphonates, which are less selective then cathepsin K inhibition. A number of cathepsin K inhibitors have already entered clinical trials as potential treatment of osteoporosis2.

Pyrazolo[1,5-a]pyrimidine is an important heterocycle and many of its derivatives are biologically active. It represents a useful scaffold in organic synthesis. Novel pyrazolo[1,5-a]pyrimidines with such structure were synthesized. (S)-Amino acids were used as starting materials and their amine group was protected with tert-butyloxycarbonyl (Boc) protecting group. Conversion to Weinreb amides and subsequent reaction with ethynylimidogen bromide afforded yrones3. These can react with dinucleophilic 5-aminopyrazoles as 1,3-dielectrophiles. Cyclization took place in methanol at room temperature and pyrazolo[1,5-a]pyrimidines were formed3. They were purified by filtration or column chromatography. Different starting compounds led to diverse pyrazolo[1,5-a]pyrimidines. 3-(Methoxycarbonyl)pyrazolo[1,5-a]pyrimidine was of particular interest since it could be hydrolyzed to carboxylic acid, which was transformed to carboxamides. Lastly Boc protecting group of some products was removed by acidolysis.

Synthesized pyrazolo[1,5-a]pyrimidines were tested for inhibition of cathepsin K. Due to fluorescent nature of many pyrazolo[1,5-a]pyrimidines, enzymatic activity was followed photometrically. The resulting data were analyzed and all inhibitors were found to act as linear inhibitors. Inhibition constants for some compounds were calculated. The best inhibition was found for compound 4, a deprotected form of pyrazolo[1,5-a]pyrimidine 3, which was synthesized from 5-aminopyrazole 1 and ynone 2. Molecular docking in the active site of cathepsin K was also carried out for all active compounds.

![Pyrazolo[1,5-a]pyrimidine synthesis](image)

References
SPECTROSCOPIC STUDIES OF MITOCHONDRIA TARGETING MULTIPLY CHARGED DABCO-CYANINE DYES

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Within the last two decades novel design of non-covalent recognition of DNA/RNA by small molecules very often combines two or more non-covalent binding modes (intercalation, groove binding, or electrostatic sugar-phosphate backbone binding) [1] aiming for new dyes selectively reporting on structural differences of DNA and RNA secondary structures. Within this context, aromatic cationic dyes with the ability to penetrate through the cell membranes are of great interest. In general, the tendency of lipophilic cationic dyes is to retain preferentially in the mitochondrial space, which renders them in particularly important group of fluorescent probes for the study of mitochondrial lipid bilayer, membrane-permeability and specific staining of these organelles.

Encouraged by our previous research efforts in design of specific mitochondrial fluorescent probes based on benzoxazolium and benzothiazolium dicationic monomethine cyanine dyes [1-3], we have prepared new series of lipophilic cyanine dyes equipped with several cationic quaternary ammonium moieties. Spectroscopic and isothermal titration calorimetry studies as well as MTT assay and subcellular localization have revealed that presented dyes combine very low cytotoxicity with efficient cellular uptake and remarkable fluorescent marking of mitochondria.

References
CC chemokine receptor 2 (CCR2) is a class A G protein-coupled receptor (GPCR) that plays a key role in the migration of leukocytes to sites of inflammation. As such, CCR2 represents a potential drug target in many inflammatory and immune diseases, such as atherosclerosis, multiple sclerosis and cancer. Yet all CCR2 antagonists developed so far have failed in clinical trials due to lack of efficacy. This makes the development of novel tools and concepts necessary to better study drug receptor pharmacology in early drug discovery phases.

In this regard, the recent crystal structure of CCR2 has suggested a new manner of pharmaceutical intervention, i.e. using intracellular allosteric modulators. In addition, irreversible or covalent probes represent important pharmacological tools that allow a variety of applications: study of drug-target binding kinetics, assist in target crystallization or study of in vivo target localization, among others. Thus, we aimed to develop and characterize an intracellular covalent probe for CCR2, as this might lead to the development of a new pharmacological tool for this receptor.

Based on the structure of a known CCR2 intracellular ligand, SD-24, we designed and synthesized several potential covalent ligands by incorporating different electrophilic groups as reactive warheads. Next, a combination of radioligand binding and functional assays allowed us to identify compound LUF7591 as an intracellular covalent binder for CCR2. In addition, in silico modeling followed by site-directed mutagenesis of CCR2 confirmed that LUF7591 binds to the intracellular pocket of CCR2, where a cysteine residue appears to be the target amino acid for the irreversible interaction.

To conclude, we report the design, pharmacological characterization and binding mode of LUF7591, a first covalent probe for CCR2. This tool compound might represent a promising approach to further study CCR2, both in vitro and in vivo.

References
INCORPORATING SUGAR AMINO ACIDS INTO LINKERS FOR PREPARING HIGHLY HYDROPHILIC ANTIBODY-DRUG CONJUGATES

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Antibody-drug conjugates (ADCs) have been developed to deliver cytotoxic agents to tumors and have the potential for increased clinical benefit to cancer patients. One of the major drawbacks of ADCs is their tendency to form the aggregates which is one of the inherent properties of high molecule weight proteins. The coupling of hydrophobic toxins onto the monoclonal antibodies further renders the ADCs to be more easily to form aggregates. To overcome this issue, we incorporated highly hydrophilic sugar amino acids onto linkers and MHT-71 was generated as one of the promising linker-toxins and used to conjugate to various monoclonal antibodies. The EGFR-targeting Erbitux-MHT-71 was prepared and characterized with D-ribose derived sugar amino acid to enhance the hydrophilicity and cathepsin B cleavable Val-Cit linkage to release the auristatins payload. The average DAR of Erbitux-MHT-71 was within 3.5–4. The in vitro cytotoxicity assay results showed that the general IC[50] of Erbitux-MHT-71 is below 0.1 nM in FaDu (HNSCC cell line) and several esophageal squamous cancer cell lines such as KYSE510, KYSE150 and KYSE30. Erbitux-MHT-71 was stable in rat and human plasma and less than 5% of toxin leaking after incubation for 3 days. The PK profile of Erbitux-MHT-71 was similar to Erbitux in rat. In mouse xenograft tumor models (FaDu and KYSE 30), Erbitux-MHT-71 showed impressive efficacy in tumor growth inhibition after a single intravenous dose of 5 mg/kg. In conclusion, Erbitux-MHT-71 having highly hydrophilic sugar amino acid moiety could enhance several properties including solubility, conjugation efficiency, stability, and efficacy.
ISOLATION OF BIOACTIVE POLYPRENYLATED TETRONIC ACID DERIVATIVES FROM HYPERICUM BARBATUM JACQ

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Hypericum species are prominent in polycyclic polyprenylated compounds, especially acylphloroglucinols, whose biological activity varies largely due to the possibilities of prenylation, cyclization and oxidation. Among other activities, antidepressant, analgesic, antioxidative, anti-inflammatory, antimicrobial and cytotoxic are predominant[1]. This increases the interest for discovery and isolation of new polyprenylated compounds. H. barbatum Jacq. is a plant distributed widely throughout Europe, from Austria to Greece, through Balkans and Mediterranean regions[2]. Since its chemical composition is poorly investigated, this plant represents an interesting source for phytochemical research.

Air-dried and powdered aerial parts of H. barbatum Jacq. were extracted with petroleum ether (PE). Dry PE extract was defatted on Diaion HP-20 column, and the most polar post-defatting fraction (eluted by 90% MeOH) was further exposed to the 1H NMR-guided fractionation using subsequent CPC and adsorption chromatography on a silica gel-based column. After final separation and purification on a reversed phase semi-preparative HPLC column, four novel polyprenylated tetronic acid derivatives were isolated – 1, 2, 3 and 4 (Fig. 1). Their structures were elucidated using 2D NMR spectroscopic data and HR-MS.

Cytotoxic activity was evaluated against HepG2 liver tumor cells, HeLa cervix epitheloid carcinoma, PC-3 prostate tumor cells, A549 lung tumor cell lines and MRC-5 healthy human fetal lung cells, with the four compounds exhibiting moderate cytotoxicity against all examined cell lines. IC50 values were 9.22–17.0 µM for HepG2, 12.8–21.8 µM to inhibit growth of HeLa cells, 14.4–27.8 µM for PC-3 tumor cells and 20.2–27.1 µM for A549 lung tumor cells. Unfortunately, they were also toxic for the healthy human fibroblast lung cell line MRC-5, with IC50 13.4–20.4 µM. Similarity in action is noted among the analogous compounds 1 and 3, as well as 2 and 4. The four examined compounds did not inhibit growth of Gram negative bacteria (Escherichia coli, Salmonella enterica subsp. enterica serovar Enteritidis and Pseudomonas aeruginosa), but showed notable activity against Gram positive bacteria. MIC values were 4.80–16.0 µg/mL for Staphylococcus aureus, 6.70–16.0 µg/mL for Bacillus subtilis subsp. spizizenii, 46.0–102 µg/mL for Enterococcus faecalis and 5.70–16.0 µg/mL for Listeria monocytogenes. The compound 3 also showed moderate antiparasitic activity against Plasmodium falciparum, Trypanosoma cruzi, T. brucei rhodesiense, and Leishmania donovani, with IC50 values of >10 µg/mL, 14.7 µg/mL, 15.2 µg/mL and 2.60 µg/mL, respectively. However, it was also toxic for the rat myoblast cell line L6, with IC50 54.5 µg/mL.

Figure 1. Tetronic acid derivatives isolated from H. barbatum Jacq.

References
DISCOVERY AND SYNTHESIS OF NOVEL CLASSES OF PATHWAY-SELECTIVE INHIBITORS THAT BLOCK NF-κB ACTIVATION BY GENOTOXIC STRESS

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Classical cancer therapies eliminate fast proliferating cells through the genotoxicity of irradiation or non-specific chemotherapeutics. A particular problem of this approach is that these tumor cells become resistant to cell death-inducing signals. The promotion of cell survival by genotoxic stress-activated IkappaB kinase (IKK)/NF-kappaB pathway has been described to play an important role in therapy resistance, and therefore this pathway is considered as a potential target in novel types of cancer therapy. Using a high-throughput screening a set of small molecule inhibitors was identified, which selectively inhibit the activation of IKK/NF-kappaB by chemotherapeutic agents or irradiation, but not by physiological cellular stimuli. Two promising hits revealed affinity within a sub-micromolar concentration range and both compounds showed stimulus-specific inhibition of IKK/NF-kappaB activation, by irradiation or DNA DSB-inducing chemotherapeutics, but not by TNFalpha or IL-1beta. These two candidates were selected for medicinal chemistry optimization. One candidate has a benocanthinone scaffold and the other one has a quinoline motive. The strategy and progress of the chemical optimization of the benzocanthinone-scaffold is presented. For the improvement of the cellular activity, novel types of substituted dihydropyrazolo[3,4-b]indoles, 5H-pyrimido[5,4-b]indoles and 5H-pyridazino[4,5-b]indoles were designed and synthesized. In addition, a small SAR study was performed to get better insight into the necessary features of the compound for the interaction with the unknown target. First biological data of the new analogues show promising results in a similar range as the initial hits and suggest a distinct NF-kappaB pathway interference.
SYNTHESIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF PHENYL CARBAMIC ACID DERIVATIVES CONTAINING AN N-ARYLPIPERAZINE SALT-FORMING MOIETY

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Designed hybrid compounds (5a-e) were prepared by a two-step synthetic process involving nucleophilic addition of 4-(trifluoromethyl)phenyl isocyanate (I) and (±)-oxiran-2-ylmethanol (2) in anhydrous toluene. The resulting (±)-oxiran-2-ylmethyl-[1-(4-trifluoro- methyl)phenyl]carbamate (3) reacted with 1-(3-/4-R substituted phenyl)piperazines (4a-e) in anhydrous propan-2-ol. Final 3-[4-(3-/4-R substituted phenyl)piperazin-1-yl]-2-hydroxy-propyl-4-(trifluoromethyl)carbamates (5a-e) were synthesized in the yields, which ranged from 78% to 85% after being recrystallized using a mixture of propan-2-ol and methanol. The derivatives 5a-e were in vitro screened against Staphylococcus aureus ATCC 2213, methicilin-resistant S. aureus 63718, vancomycin-resistant Enterococcus spp. (342 B, 368 and 725, respectively), Escherichia coli ATCC 25922, Mycobacterium smegmatis ATCC 700084, M. kansasii DSM 44162 as well as against Candida albicans CCM 8186, C. krusei CCM 8271 and C. parapsilosis CCM 8260, respectively. The molecules 5j and 5k containing chlorine atom(s) within a salt-forming moiety were considered the most effective.

Keywords: synthesis, N-arylpiperazines, antimicrobial activity

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF DAVIDSON-LIKE IMIDAZOLE DERIVATIVES

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The modification of amino acids leads to valuable building blocks for the synthesis of bioactive compounds. By keeping the amino group protected, the carboxylic acid functionality can be converted in two steps to an imidazole moiety via Davidson-like heterocyclization. This reaction allows for a combinatorial approach, in which two positions at the heterocycle can be modified. Davidson-like imidazole syntheses have been successfully utilized for the preparation of sphingosine-1-phosphate analogues [1], antimalarial imidazolopiperazines [2, 3], tetrahydro-beta-carboline-type somatostatin antagonists [4] and to generate linker structures in antiplasmodial 7-chloro-4-aminoquinoline derivatives [5].

Herein, we report on the synthesis of imidazole derivatives by using N-protected cyclohexylalanine as the starting material, which was consecutively converted to appropriate esters and subjected to a Davidson-like heterocyclization. By using different α-haloketones, two points of diversity were introduced at position 4 and 5, respectively. We have investigated the three-dimensional structure of a disubstituted (R1,R2 ≠ H) and a monosubstituted (R2 = H) imidazole derivative by means of X-ray diffraction analysis. The heterocyclic compounds have been biologically investigated as inhibitors for human neutrophil elastase.

References
DESIGN AND SYNTHESIS OF POTENTIAL ALLOSTERIC INHIBITORS OF TISSUE TRANSGLUTAMINASE

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Tissue transglutaminase (TG2), the most ubiquitous member among the human transglutaminase enzyme family, is responsible for various modifications to proteins, particularly cross-linking of proteins into large molecular weight polymers that are more resistant to degradation. Under stress, adventitious cross-linking due to overexpression of TG2 has been implicated in numerous diseases such as celiac disease, fibrosis, neurodegenerative disorders and cancer. Therefore, TG2 is an ideal target for the development of potent, selective inhibitors with acceptable toxicity. Among TG2 inhibitors, irreversible inhibitors have been the most widely developed but their further progress in clinical trials is prevented due to potential toxicity. Our research has focused on the development of a new series of allosteric TG2 inhibitors containing no reactive functionality based on the structure of a lead allosteric inhibitor LDN-27219 [1, 2]. Computational modelling techniques including protein-ligand docking and molecular dynamic simulations have been used to identify the presumed allosteric site and then to design small molecules with optimal fit into the site. A series of potential allosteric inhibitors have been successfully synthesised, characterised and is about to be screened in vitro against TG2.

References
A COVALENT ANTAGONIST FOR THE HUMAN ADENOSINE A3 RECEPTOR

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The human A3 adenosine receptor (hA3R) plays an important role in both physiological and pathophysiologic conditions, such as cell proliferation, cell differentiation, neuroprotection, cardioprotection and apoptosis. In the past we have searched for potent and selective hA3R antagonists, leading to a set of structurally diverse antagonist classes. In particular, tricyclic xanthine derivatives of 1H,3H-pyrido[2,1-f]purine-2,4-dione have been reported to exert high affinity and selectivity for hA3R. Building on these results, we report an analog, LUF7602, equipped with a reactive electrophilic fluorosulfonyl functionality, as a selective covalent antagonist of hA3R.

In a radioligand binding assay this ligand acted as a potent antagonist, with an apparent affinity for the hA3R in the nanomolar range. Its apparent affinity increased with longer incubation time, suggesting an increasing level of irreversible binding over time. An in silico hA3R-homology model was used to study the binding mode, indicating that a tyrosine residue Y2657.36 was responsible for the covalent bond formation. Site-directed mutagenesis was performed to demonstrate that the amino acid residue was the unique anchor point of the covalent interaction. Subsequently, LUF7602 was tested in [35S]GTPγS functional assays. Preincubation with LUF7602 caused a concomitant decline in the agonist's maximal response, indicating insurmountable antagonism, another proof of the covalent receptor labeling. In contrast, coincubation with this antagonist generated a parallel rightward shift of the agonist's concentration-effect curve with no alteration of the maximal effect, suggesting the insurmountable antagonism was competitive, due to an irreversible blockade to reduce the total receptor population available.

All these data contribute to a better understanding of the covalent interaction between LUF7602 with the receptor. This covalent antagonist may serve as a valuable molecular translational tool for further investigating the role of hA3R in different pathophysiological conditions.

References
N-ACETOHYDROXAMIC ACID DERIVATIVES AND N-HYDROXYIMIDES AS METAL-CHELATING AGENTS AGAINST VIRAL AND PARASITIC DISEASES


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Influenza is an acute respiratory disease that is responsible for substantial morbidity and mortality in humans and causes seasonal outbreaks and sporadic pandemics. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are among the most common blood borne pathogens and both viruses can lead to a chronic ongoing infection, which can progress to cirrhosis and hepatocellular carcinoma. Human African Trypanosomiasis and Chagas disease are neglected diseases that range in severity from comparatively mild to near invariably fatal in developing countries. Nowadays, poor efficacy and high toxicity of the available drugs, as well the increasing number of drug-resistant pathogens render the treatment even difficult for the aforementioned infections. Thus, there is an utmost need for new classes of therapeutics.[1]

Metalloproteins are responsible for a wide variety of essential viral and parasitic functions, and consequently, they have garnered interest as potential therapeutic targets. The importance of the metalloenzymes, along with the fact that some of them have no counterparts in the host cell, prompted the development of novel scaffolds, bearing a metal-chelating motif, as potent inhibitors.

Utilizing molecular simulation tools and findings in the literature, we have rationally designed and synthesized two new classes of metal-chelating agents (N-acetohydroxamic acid derivatives and N-hydroxyimides)[2][3] which coordinate bidentaly metal ions to the metalloenzyme’s active site. The novel compounds were tested for their antiviral and antiparasitic activity, and they were considerably potent with IC50 values in the low nanomolar range; moreover, promising safety profiles were detected. Additional theoretical studies and docking calculations will contribute to acquire more structure-activity relationship (SAR) data, offering to the design of more agents with broad-spectrum activity.

References
Nowadays, the use of natural compounds with confirmed biological activity in designing new dietary supplements is becoming more and more popular. Natural compounds due to their origin are considered as GRAS and gain greater trust of consumers and patients. Although many natural compounds, exhibit high biological potential in *in vitro* cytotoxic studies their activity usually decreases when used in human trials which is explained by their limited bioavailability and very complex metabolism of human body.

Lipid nanocarriers are promising tool for delivery of biologically active compounds (BACs) that enhance their stability and bioavailability. The present work has been carried out to study the potential of mixed lipids and their proportions on formation and properties of nanostructured lipid carriers (NLCs). pure isomers (c9,t11 and t 10,c12) of conjugated linoleic acid were used as an biologicaly active component (BAC) of lipid phase together with Gelucire® 43/01 (GLCR-43) and Mygliol® 812 N stabilized by a mixture of Tween® 80 (T80) and soybean phosphatidylcholine. Lipid nanoparticles were prepared by the high shear homogenization fallowed by ultrasonication method. The mean particle size, polydispersity and zeta potential of NLC were were characterized by dynamic light scattering (DLS). Morphology was determined by TEM and revealed fairly spherical shape of nanoparticles. Both types of nanocarriers were subjected to stability study and were stored either in 4, 20 or 40°C for 7 days after that DLS and Lumisizer® analyses were performed. All samples stored in 4°C were bigger in size and polydispersity but also more stable during centrifugation. Lumisizer® analysis showed creaming phenomenon in all nanocarriers formulations. Instability index evaluated for the centrifugation time of 3 h was lower for samples stored for 7 days in 4°C whereas samples kept in 20 and 40°C showed higher instability index.
SYNTHESIS OF FLUORESCENT MOLECULAR PROBE FOR SCREENING POTENTIAL INHIBITORS OF HUMAN DECCAPING SCAVENGER (HDCPS)

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The 5′ mRNA cap protects mRNA from 5′ to 3′-exonuclease degradation and has an important role in gene expression processes including translation initiation and mRNA turnover. The cap interacts with several proteins involved in mRNA metabolism which have often been linked to disease development. Therefore, synthetic cap analogues are useful in wide range of applications, including modulating activity of key cap-dependent enzymes. One of them is Decapping Scavenger (DcpS) – pyrophosphatase cleaving the triphosphate chain within the cap to release m7GMP and a 5′-diphosphate from the rest of the molecule, thus degrading cap structure following mRNA 3′ to 5′ decay. DcpS is also a molecular target in Spinal Muscular Atrophy (SMA) treatment, thus its inhibitors are potential therapeutic agents. Fluorescent molecular probes that bind DcpS with high affinity can be used to find tightly binding inhibitors of DcpS as potential therapeutics for SMA. Here, we designed and synthesized a fluorescently labelled mRNA cap analogue with resistance and high binding affinity to DcpS (Figure). Key modification to achieve both features was phosphorothiolate group neighbouring 7-methylguanosine moiety. Carboxyfluoresceine dye was attached to the base of second nucleoside via diamine linker using NHS chemistry. The preliminary spectroscopic and biochemical characteristics of the probe and its applications for search of DcpS inhibitors by fluorescence polarization method also will be presented.

References
DESIGN, SYNTHESIS AND TESTING OF THE PLASMODIUM FALCIPARUM DIHYDROOROTATE DEHYDROGENASE INHIBITORS

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Malaria is a third world disease that annually causes around 200 million infections and about half a million deaths.

The biggest issue is the rapid development of resistance to all newly approved medicines, which means that malaria is still an incurable disease. The main cause of infection is the Plasmodium falciparum parasite, which can be transmitted through mosquitos of the Anopheles type.

Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH), a fourth enzyme in the de novo pyrimidine biosynthesis pathway has emerged as a promising target for antimalarial drugs. PfDHODH is located on the outer side of the inner mitochondrial membrane and catalyzes the conversion of dihydroorotate to orotate. The reaction requires two cofactors, a flavinmononucleotide (FMN), which is needed for the oxidation of dihydroorotate and ubiquinone, a terminal electron acceptor, necessary for the reoxidation the FMN.

In Plasmodium falciparum the path of de novo pyrimidine biosynthesis is the only source of pyrimidine production, whereas in humans this biosynthetic pathway is only one of the sources of pyrimidines, which makes PfDHODH a promising target. Studies have shown that PfDHODH inhibition leads to parasite death.

In an effort to discover new and potent PfDHODH inhibitors, a number of different compounds were virtually tested using the Schrödinger Glide molecular docking program. The best results were obtained with two types of compounds, bicyclic 3-pyrazolidinones and theophylline-7-acetamides. Bicyclic 3-pyrazolidinones were prepared by a microwave-assisted three-component reaction between a 3-pyrazolidinone, an aldehyde, and an acrylate via formation of an azomethine imine, followed by 1,3-dipolar cycloaddition. Teophylline-7-acetamides were prepared by condensation of easily available theophylline 7-acetic acid with α-amino esters, followed by hydrolysis of the ester group.

The recombinant enzyme PfDHODH was expressed by DH5α E. coli cells. After the expression the enzyme was isolated and purified with nickel affinity chromatography.

The assays of biological activity were performed by measuring the absorbance of the colorimetric substrate dichlorophenolindophenol (DCIP). Results show IC50 values for compounds 1–4 in the range of low μM concentrations (Figure 1).

The selectivity against Homo sapiens dihidroorotate dehydrogenase (HsDHODH) was tested. Bicyclic 3-pyrazolidinones show better selectivity compared to theophylline-7-acetamides.

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NOWAYS, antimicrobial resistance is a global threat to public health. This well know plague only recently burst out, prompting to the urgent need of developing efficient antibiotics with innovative mechanisms of action.

In this context, the bacterial divisome turned out to be an interesting and promising target (1). Cell division proteins are indeed crucial for bacteria viability, are widely conserved among several species and are completely absent in eukaryotic cells, thus strengthening the selectivity of the novel antimicrobics. FtsZ (Filamentous temperature sensitive Z) is one of the essential cell division proteins; FtsZ is a tubulin homologue (2) and is the first protein that localizes to the mid-point of the cell and undergoes polymerization in a GTP-dependent manner, bringing to the formation of the Z-ring. It recruits at least ten other cell division proteins, which enable cell constriction, the formation of mesosome and two daughter cells (3).

Recently, we studied and developed FtsZ inhibitors, starting from the most significant results of other research groups and confirming that FtsZ inhibition results in a bactericidal effect.

We prepared 3-Methoxybenzamide (3-MBA) derivatives, structurally similar to the FtsZ inhibitors lead compound: PC190723 (4-6).

Our derivatives (which general structure is depicted above) were designed replacing the thiazolopyridine of PC190723 with differently substituted 1,4-benzodioxane or 1,4-benzoxathiane. We further assessed the Structure Activity Relationship (SAR) of this class, through a series of isosteric, positional or substituent modifications (7-9).

These molecules proved to strongly inhibit S. aureus, E. faecalis and M. tuberculosis viability and to target FtsZ. We specifically performed two different biochemical assays, aimed at studying GTPase and polymerization activities of S. aureus FtsZ, when incubated with our compounds.

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In 2014, GSK performed a high-throughput-screen of 1.8 million compounds against three kinetoplastid parasites. This data was published as an open source in an effort to encourage research and drug development for these neglected diseases. A simple arylthioether compound (compound 1) was found to have desirable activity against \textit{Trypanosoma cruzi} (the parasite responsible for Chagas disease) and was selected as the hit compound for this project. The initial investigation led to the discovery of an even more potent compound, with a superior pIC\textsubscript{50} of 7.5 (compound 2).

This class of compounds showed promising results in acute \textit{in vivo} efficacy studies and even more potent compounds have been developed since. However, several issues have been identified for this chemical series, such as toxicity and low oral exposure. A full toxicity study was undertaken and several alerts were identified that relate to CNS and cardiovascular toxicity. In order to address these concerns, future analogues have been focused to decrease toxicity and increase exposure. This will be achieved by exploring lipophilicity, solubility and increasing microsomal stability.

References

AN INFRARED STUDY OF CONCANAVALINE A AGGREGATION AT pH 5 AND 9

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Abstract. We presented the application of infrared spectroscopy to study protein aggregation. The aggregation of Concanavaline A was induced by heating the protein solution at two different pH values. We showed that properly processed infrared spectra provide valuable information about the protein structure and structural changes during the process of aggregation. It is shown, that infrared spectroscopy offers an opportunity to distinguish between two similar and related processes; aggregation and fibrillation. Moreover, difference spectra provide details about hydrogen bonding.

Keywords: Concanavaline A, aggregation, fibrillation, infrared spectroscopy, difference spectroscopy, Amide I, Amide III
Telomerase is a ribonucleoprotein enzyme which protects the ends of chromosomes from excessive shortening during cell division through the ability to synthesize telomeres repeats. This preservation ensures lack of degradation of chromosomes and the consequent induction of replicative senescence or apoptosis. The telomerase is detected in about 85% of tumors, whereas in normal human somatic cells expression is absent or is significantly lower. This unique hallmark gives rise to use this enzyme as a target in drug research.

Therefore, previous studies show increasing concern about interest in research for new telomerase inhibitors. It needs to be highlighted that some natural products, commonly derived from plant materials, play a vital role in disrupting the structure of telomeres. From a wide range of compounds, some methylxanthines derivatives have been considered in this approach. A negative correlation between caffeine intake and the shortening of telomeres in humans may indicate that this compound inhibits telomerase. Therefore, the effects of caffeine and pentoxifylline as another its derivative were determined in these studies on telomerase activity in lung carcinoma cells.

A better understanding of natural product’s molecular activity can contribute to the conceiving of a pharmacophore for the design and synthesis of new drugs. Moreover, plant metabolites as potential drugs can be intake as a functional food by the patients in the treatment or prevention of cancer.
Flavonoids are a large group of naturally occurring phenolic compounds, present in fruits, vegetables, teas and wine, which possess broad-spectrum of pharmacological activities and extensive biological effects. Therefore, flavonoids are an attractive source for drug discovery. Their potential to act as anti-tumourigenic and anti-proliferative agents has been reported previously but is not yet fully understood. In vitro earlier studies indicate that flavonoids have been to exert their anti-cancer effects at different stages of cancer development and inhibit cellular proliferation, induce cellular cytotoxicity by modulating mitogenic and apoptotic signaling pathways, and affect cell-cycle. Checkpoints at both G1/S and G2/M of the cell cycle in cultured cancer cell lines have been found to be perturbed by flavonoids. Furthermore, targeting human telomeric G-quadruplex DNA also could be one of the mechanisms by which flavonoids exert anticancer activity.

The purpose of the current study was to evaluate the effects of selected flavonoids on the proliferation, apoptosis, and cell cycle of different human cancer cells: MDA-MB-231 (estrogen-negative breast carcinoma cells), MCF-7 (estrogen-positive receptor breast carcinoma cells), A549 (adenocarcinomic human alveolar basal epithelial cells) and HL-60 (human leukemia cell line).
CELL-DENSITY DEPENDENCE OF CYTOLOGICAL STAGE PROFILE: A NOVEL APPROACH TO SCREENING FOR CYTOSTATIC AGENTS ACTIVE TOWARDS LEUKEMIC STEM CELLS

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Leukemic Stem Cells (LSCs) are considered necessary to sustain leukemia proliferation and expansion. Furthermore, their multidrug resistance is a major reason for Acute Myeloid Leukemia (AML) treatment failure and relapses. These properties of LSCs create a need for a high-throughput screening platform allowing selection of compounds active towards these cells.

We found that prolonged cell culture under conditions of strict density control alters cytological stage profile. Using this approach, three HL-60 sublines were generated, each with characteristic stage profile manifested in morphology, expression of surface markers and redox-state proteins as well as mitochondrial potential.

In particular, low cell culture density increased the fraction of primitive cells (which include LSCs). Interestingly, a subline with similar stage profile was obtained by a 240-day treatment with anthrapyridazone BS-121. HL-60 sublines generated in this work served as a screening platform for anthrapyridazones. Out of all tested compounds, C-123 was found the most potent against primitive cell stages. It exhibited low toxicity in vivo weakly affecting blood morphology of healthy mice. Furthermore, it generated relatively low amounts of Reactive Oxygen Species (ROS). The properties of C-123 suggest a potential application in AML treatment. Specifically, it could find use in conditioned myeloablation preceding allogeneic bone marrow transplant or ex vivo treatment preceding autologous bone marrow transplant.

Cell-density dependence of cytological stage profile could be used to construct patient-specific screening platforms allowing search for drugs effective in case of potential AML relapse.
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