MMCS 2019

FACING NOVEL CHALLENGES IN DRUG DISCOVERY

2nd Molecules Medicinal Chemistry Symposium

BARCELONA | SPAIN | 15 – 17 MAY 2019

Program and Abstract Book

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2nd Molecules Medicinal Chemistry Symposium: Facing Novel Challenges in Drug Discovery

AXA Convention Centre

Barcelona, Spain

15 – 17 May 2019

Conference Chair

Prof. Dr. Diego Muñoz-Torrero

Conference Co-Chair

Prof. Dr. F Javier Luque

Organised by

Conference Secretariat

Sara Martínez

Facundo Santomé

Lucia Russo

Pablo Velázquez

Jiahua Zhang

Judith Wu

#MMCS2019

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MMCS2019 – Facing Novel Challenges in Drug Discovery
15 – 17 May 2019, Barcelona, Spain

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	Session 1. Part 1	*Session 4*	*Session 1. Part 2*
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Wednesday 15 May 2019: 08:00 - 12:45 / 14:30 - 18:30

Thursday 16 May 2019: 08:45 - 13:00 / 14:30 - 16:00 / **Conference Dinner: 20:00**

Friday 17 May 2019: 08:30 - 12:30 / 14:00 - 18:15

Conference Programme

Wednesday 15 May 2019

08:00	Registration Desk Open (Check-in)
08:45 – 09:00	Opening Ceremony Chairs: Diego Muñoz-Torrero and F. Javier Luque

Session 1. Part 1
Molecules against Cancer
Chairs: Diego Muñoz-Torrero and F. Javier Luque

09:00 – 09:30	**Stephen Neidle** "The Targeting of Quadruplex Nucleic Acids in Human Cancers"
09:30 – 09:45	**Seyed A. Tabatabaei Dakhili** "FOXM1 Inhibitors: Emergence of a Neglected Binding Force"
09:45 – 10:00	**Simona Sestito** " Novel Dual PDK1/AurK-A Inhibitors for Cancer Therapy: Med Chem Evolution and Crystallographic Investigation"
10:00 – 10:15	**David Barker** "Development of Thienopyridines as Potent Antiproliferative Agents"
10:15 – 10:30	**Konstantin Volcho** "Tdp1 Inhibition as a Promising Approach to New Anticancer Drugs"
10:30 – 11:15	**Coffee Break**

Session 1. Part 1
Molecules against Cancer
Chair: Katalin Prokai-Tatrai

11:15 – 11:45	**Eva Estébanez-Perpiñá** "Translating Androgen Receptor Structure into Precision Medicines"
11:45 – 12:00	**Wen-Wu Li** "Synthesis and Evaluation of Thymoquinone Analogues as Anti-ovarian Cancer Agents "
12:00 – 12:15	**Anna M. Costa** " Amphidinolides and Iriomoteolides, Potent Anticancer Macrolides"
12:15 – 12:30	**Beata Morak-Młodawsk** " Novel Dipyridothiazines with 1,2,3-Triazole Substituents—Synthesis and Anticancer Activities"
12:30 – 12:45	**Maria Cristina De Rosa** “Discovery of a Selective NEK6 Kinase Inhibitor by Virtual Screening”

12:45 – 14:30	**Lunch**
13:30 – 14:15	***Workshop "Visual Ideation & Decision Assistance for Every Drug Researcher" by BioSolveiT***

Session 2

Targeting Protein Degradation in Drug Discovery

Chair: Simona Collina

14:30 – 15:00	**Yi Sun** "Sag/Rbx2 E3 Ubiquitin Ligase: from Target Validation to Drug Discovery"
15:00 – 15:30	**Alessio Ciulli** "Targeted Protein Degradation with Small Molecules: How PROTACs Work"
15:30 – 15:45	**Carles Galdeano** "Expanding the Toolbox of E3 Ligases for Protein Degradation: Targeting the "Undruggable" Fbw7 E3 Ligase"
15:45 – 16:00	**Philip Ryan** "Design, Synthesis, and Biological Evaluation of Bimodal Glycopeptides as Inhibitors of Neurotoxic Protein Aggregation"
16:00 – 16:15	**Imane Bijij** "Tracing Potential Covalent Inhibitors of an E3 Ubiquitin Ligase Through Target-focused Modelling"
16:15 – 16:30	**Tiantian Xu** "Structural-Based Virtual Screening to Identify a Class of Small Molecules that Selectively Inhibits Cul-5 Neddylation"
16:30 – 17:00	**Coffee Break**

Session 3

New Avenues in Kinetic Target-Guided Synthesis

Chair: Rino Ragno

17:00 – 17:30	**Jörg Rademann** "How Proteins Catalyze Ligand Formation: Protein-Templated Fragment Ligation Employed in the Validation of Cancer Targets"
17:30 – 18:00	**Rebecca Deprez-Poulain** "Kinetic Target-Guided Synthesis as a Tool for Drug-Discovery: Successes, Challenges and Applications to Metalloproteases"
18:00 – 18:30	**Selected Posters 3-min Flash Presentations: Bosch** (Poster No. 43); **Gizynska** (30); **Gladysz** (34); **Jumde** (58); **Kaminski** (80); **Morales** (53); **Mousavifar** (71); **Pons** (67); **Ribić** (51); **Zhang** (65).

Thursday 16 May 2019

Session 4
In the Pursuit of Novel Drugs and Molecular Targets
Sponsored by the IBUB – Drug and Target Discovery
Chair: Marçal Pastor-Anglada

08:45 – 09:15 **Xavier Barril** "Targeting Novel Allosteric Sites with Confidence: Methods and Applications"
09:15 – 09:30 **Katalin Prokai-Tatrai** "CNS-selective Estrogen Therapy"
09:30 – 09:45 **Yanan Li** "Discovery of Small Molecule Inhibitors that Disrupt the Interaction of Neddylation E1 NAE and E2 Ube2F for Cancer Therapy"
09:45 – 10:00 **Jessica Holien** "Drugging the Undruggable: Inhibiting MYCN Signaling"
10:00 – 10:15 **Janna Ehlert** "Novel Antimalarial Inhibitors that Specifically Target the Invasion Motor Protein Myosin A in Malaria Parasites"
10:15 – 10:30 **Laura Castilla-Vallmanya** "Understanding the Pathophysiology and Searching for Biomarkers for Rare Genetic Developmental Diseases"

10:30 – 11:30 **Coffee Break**
10:30 – 10:40 ***Conference Group Photograph***

Session 4
In the Pursuit of Novel Drugs and Molecular Targets
Chair: Federico Gago Badenas

11:30 – 12:00 **Barry Potter** "Steroid Sulfatase Inhibition: From Concept to Clinic and Beyond" - *Molecules* 2018 Tu Youyou Award Winner
12:00 – 12:15 **George Kokotos** "Inhibitors of Phospholipid-Hydrolyzing Enzymes as Novel Agents against Pulmonary Fibrosis and Diabetes Type-1"
12:15 – 12:30 **Yun Shi** "How Size Matters: Designing Diverse Fragment Libraries for Novel Drug Discovery"
12:30 – 12:45 **Stefan Ilic** "Using a Combination of Fragment-Based and Virtual Screening to Discover Inhibitors for DnaG Primase of Mycobacterium tuberculosis"

12:45 – 13:00 **Leire Iralde Lorente** "Small Molecules as Potential Inhibitors of the 14-3-3/c-Abl Interaction for the Treatment of CML"

13:00 – 14:30	**Lunch**
13:30 – 14:30	***Poster Session A***

Session 4

In the Pursuit of Novel Drugs and Molecular Targets

Chair: Barbara Malawska

14:30 – 15:00	**Santiago Vázquez** "Adamantane Analogs: From Anti-Influenza Drugs to Soluble Epoxide Hydrolase Inhibitors for Acute Pancreatitis"
15:00 – 15:15	**Sonsoles Velázquez** "4,4-Disubstituted N-benzylpiperidines: A Novel Class of Fusion Inhibitors of Influenza Virus H1N1 Targeting a New Binding Site in Hemagglutinin"
15:15 – 15:30	**Lisa Pilkington** "Development of Novel, Potent Phosphatidyl–Choline-Specific Phospholipase C Inhibitors"
15:30 – 15:45	**Bhautikkumar Patel** "Design, Synthesis, and Biological Evaluation of Nucleoside Analogues Acting against *Neisseria Gonorrhoeae*"
15:45 – 16:00	**Ravi Munuganti** "Targeting Neuronal Transcription Factor BRN2 in Neuroendocrine Tumors"

17:00	**Guide visit to Sant Pau** (Bus pick-up at 16:30)
20 :00	**Conference Dinner**

Friday 17 May 2019

Session 1. Part 2
Molecules against Cancer
Chairs Catherine Guillou

08:30 – 09:00	**Emília Sousa** "Strategies to Discover p53 Activators and a p73 Activator for Neuroblastoma"
09:00 – 09:15	**Roman Dembinski** "Organometallic Nucleosides: Synthesis and Biological Evaluation of Substituted Dicobalt Hexacarbonyl Alkynyl Modified 2'-Deoxyuridines"
09:15 – 09:30	**Concepción Alonso** "Synthesis of Heterocyclic Fused [1,5]naphthyridines by Intramolecular HDA Reactions"
09:30 – 09:45	**Cristina Maccallini** "Inhibitors of the Inducible Nitric Oxide Synthase as Antiglioma Agents"
09:45 – 10:00	**Janusz Rak** "Why Does the Type of Halogen Atom Matter for Radiosensitizing Properties of 5-substituted 4-thio-2'-deoxyuridines?"
10:00 – 11:00	**Coffee Break**

Session 1. Part 2
Molecules against Cancer
Chair: George Kokotos

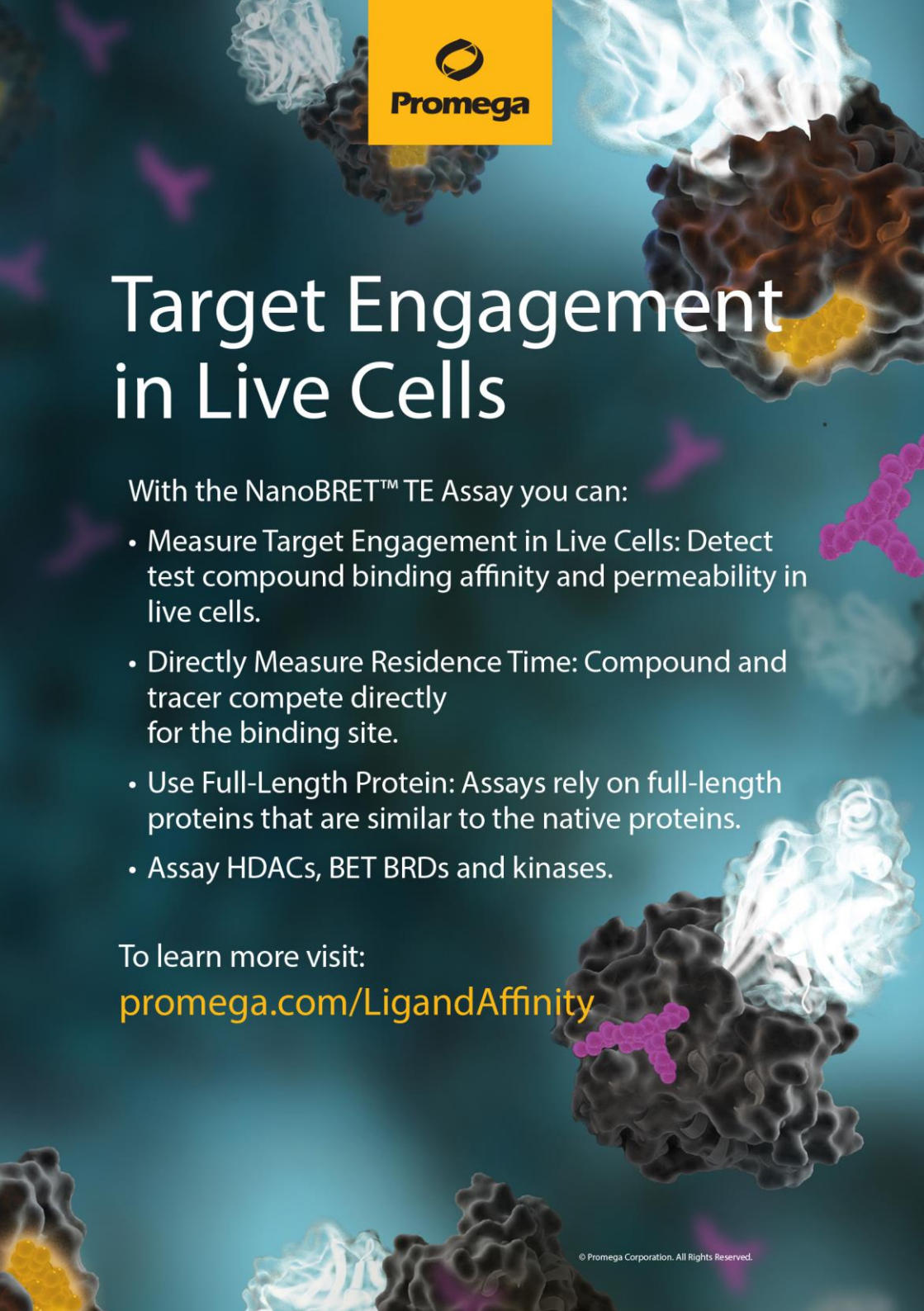
11:00 – 11:30	**Viranga Tillekeratne** "In Search of Selectivity: Design, Synthesis and Biological Evaluation of New Classes of HDAC Inhibitors"
11:30 – 11:45	**Eduard Mas** "Interaction of Epigenetically-Modified Natural Nucleosides with Membrane Transporters as a Clue to Understand their Mechanism of Action"
11:45 – 12:00	**Niv Papo** "Engineering Affinity, Specificity, and Stability in Protein Therapeutics"
12:00 – 12:15	**M. Isabel Matheu** "Synthesis of Polyfluorinated KRN7000 Analogues and Biological Implications"
12:15 – 12:30	**Claudia Cardozo** "Use of a Zebrafish Model to Evaluate Toxicity of Schiff Base Complexes of Copper (II) and Zinc (II) as Possible Antineoplastic Agents"
12:30 – 14:00	**Lunch**
13:00 – 14:00	***Poster Session B***

Session 5
Medicinal Chemistry Tales
Chair: Maria-Laura Bolognesi

14:00 – 14:30	**Christa Müller** "Tools and Drugs for Purine-Binding Targets - Important Players in Inflammation and Cancer"
14:30 – 14:45	**Maria João Matos** "Facing Novel Challenges in Neurodegenerative Diseases Drug Discovery: From Small Molecules to Targeted Therapies"
14:45 – 15:00	**William Donaldson** "Potent and Selective Estrogen Receptor-Beta Agonists which Enhance Memory Consolidation in an Ovariectomized Mouse Model"
15:00 – 15:15	**Serena Della Volpe** "Towards the Modulation of RNA-Binding proteins: New Compounds Targeting Protein HuR"
15:15 – 15:30	**Lena Trifonov** "Structurally Simple, Readily Available Peptidomimetic1-Benzyl-5-methyl-4-(n-octylamino) pyrimidin-2(1H)-one Exhibited Efficient Cardioprotection in a Myocardial Ischemia (MI) Mouse Model"
15:30 – 16:30	**Coffee Break**

Session 5
Medicinal Chemistry Tales
Chair: Rui Moreira

16:30 – 17:00	**Mark von Itzstein** "A Novel Series of Sialic Acid-Based Influenza Virus Inhibitors that Target Influenza Virus Neuraminidase"
17:00 – 17:15	**Marco Catto** "Repositioning of Dantrolene as a Multitarget Agent for Neurodegenerative Diseases"
17:15 – 17:30	**Megan Meuser** "Kinetic Characterization of Novel HIV-1 Entry Inhibitors: Discovery of a Relationship between Off-Rate and Potency"
17:30 – 17:45	**Florenci González Adelantado** "Design and Synthesis of Cysteine Proteases Inhibitors"
17:45 – 18:00	**Teodora Bavaro** "Rational Design, Synthesis and Characterization of Glycoconjugates as Potential Vaccines against Tuberculosis"
18:00 – 18:15	**Rino Ragno** "www.3d-qsar.com: A Portal to Build 3-D QSAR Models"
18:15 – 18:30	**Closing Remarks and Awards Ceremony**



Welcome by Diego Muñoz-Torrero and F Javier Luque

Dear Colleagues,

It is with great pleasure that we welcome you to the second Molecules Medicinal Chemistry Symposium (MMCS2019): Facing Novel Challenges in Drug Discovery, organized in Barcelona by MDPI, the publisher of the open-access journal Molecules. The conference follows the very successful one-day meeting, Molecules Medicinal Chemistry Symposium (MMCS): Emerging Drug Discovery Approaches against Infectious Diseases, held in September 2017 at the School of Pharmacy and Food Sciences of the University of Barcelona, which turned out to be a very fruitful forum for discussion on the specific topic of anti-infective drug discovery, in an excellent atmosphere created by the more than 70 attendees from 18 countries.

Encouraged by the great success of the initial small-format monographic MMCS2017, the three-day MMCS2019 has naturally grown by addressing a larger audience and essentially all topics of medicinal chemistry. The MMCS2019 is organized into a number of thematic sessions on medicinal chemistry of particularly challenging diseases, novel and revisited drug discovery approaches, and medicinal chemistry stories about recently implemented projects in any area not covered in the other sessions, from target and hit identification to hit-to-lead optimization, tuning of physicochemical and pharmacokinetic properties, preclinical and clinical development, etc.

Two prominent and inspiring keynote speakers per session will share the program with a number of selected oral communications. The program will be complemented by poster sessions, including a special session where 10 posters selected for the Best Poster Award will be presented in a 3-min flash-format, and social events on the second day of the conference.

We hope that you all have a great experience and enjoy your stay in Barcelona!

Diego Muñoz-Torrero
Conference Chair

Editor-in-Chief of the Medicinal Chemistry Section of *Molecules*. University of Barcelona, Spain

F Javier Luque
Conference Co-Chair

Editorial Board Member of the Medicinal Chemistry Section of *Molecules*. University of Barcelona, Spain

Molecules (ISSN 1420-3049, CODEN: MOLEFW) is the leading international peer-reviewed open access journal of chemistry, published semi-monthly online by MDPI. Originally conceived as a forum for papers on synthetic organic chemistry and natural product chemistry, *Molecules*, like the field, has evolved over its 20 years, with increasing numbers of papers on more theoretical subjects, physical organic chemistry, nanomaterials and polymer chemistry, and applied studies. Today, *Molecules* provides an advanced forum for science of chemistry and all interfacing disciplines.

Among other databases, *Molecules* is indexed by the Science Citation Index Expanded (Web of Science), MEDLINE (PubMed), and Scopus.

Journal Webpage: www.mdpi.com/journal/molecules
Impact factor: **3.098** (2017); 5-Year Impact Factor: 3.268 (2017)

General Information

MDPI, the Multidisciplinary Digital Publishing Institute, is an academic open access publisher, established in 1996. We publish over 200 peer-reviewed open access journals across ten different subject areas and offer publishing-related initiatives to scholars:

• Sciforum - A platform for academic communication and collaboration where scholars can set up free scientific conferences or participate in discussion groups.

• Preprints - A multidisciplinary not-for-profit platform for rapid communication of research results before peer-review.

• JAMS - A complete manuscript submission system that incorporates all steps from initial submission to publication, including peer-review.

• IOAP - We also offer an Institutional Open Access Program for universities and their libraries where affiliated authors benefit from discounts for publishing with our open access journals. Over 550 universities, societies and funders have joined MDPI's program since it was launched in 2013.

If you would like more information about open access or any of our services listed above, be sure to talk to us at the MDPI booth. See you there!

2nd Molecules Medicinal Chemistry Symposium – Facing Novel Challenges in Drug Discovery will be held at the AXA Convention Centre, Barcelona, on 15 – 17 May 2019.

This conference seeks to gather together experts in the field of medicinal chemistry, and aims to provide a forum for discussion regarding recent innovative medicinal chemistry projects, particularly (but not only) on the design and development of novel anticancer drugs, protein degradation inducers, target-guided synthesis of bioactive compounds, and new targets and drug candidates.

Conference Venue

Auditorium AXA of the AXA Convention Centre
Avinguda Diagonal, 547, 08029 Barcelona, Spain

Registration Desk

The desk for registration, information and distribution of documents will be open from 08:00 on 15 May 2019.

Certificate of Attendance

Upon request, the participants of the event will receive an electronic Certificate of Attendance by email once the event is concluded.

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during all scientific and social events. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accident, loss, or damage to private property incurred as a result of participation in the *2nd Molecules Medicinal Chemistry Symposium*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

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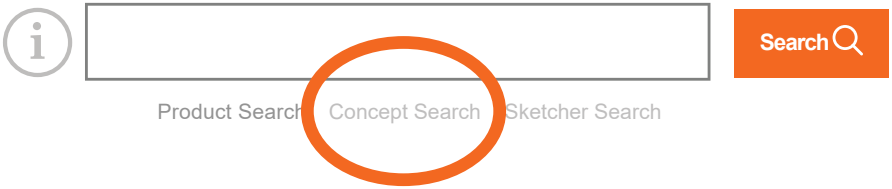

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Barcelona and Catalonia

Catalonia has become one of the favourite tourist destinations of Spain, mainly because of Barcelona, a city that never sleeps and knows how to please the big majority. With a history among the oldest in Europe, Barcelona offers a mixture of inland and seaside charms that panders the interests of everybody. The variety of artistic treasures, Romanesque churches and the works of famous artists such as Dalí, Gaudí, Miró or Picasso will make of your visit to the city a remarkable experience.

Parc Güell (Source: www.viajero-turismo.com)

Barcelona is the capital and largest city of Catalonia and Spain's second largest city, with a population of over one and half million people (over five million in the whole province). This city, bathed by the Mediterranean Sea, has become one of most cosmopolitan cities of Europe which has transformed it into the very modern, yet incredibly old city.

This beautiful city is full of what European cities are known for (outdoor markets, restaurants, shops, museums and churches) and which makes it the perfect scenario to get lost in its picturesque streets and avenues. Moreover, Barcelona's extensive and reliable Metro system will take you to more far-flung destinations. The core centre of the town, focused around the *Ciutat Vella* ("Old City"), provides days of enjoyment for those looking to experience the life of Barcelona while the beaches the city was built upon provide sun and relaxation during the long periods of agreeably warm weather. [Source: www.wikitravel.org].

Plaza Espanya (Source: www.viajero-turismo.com)

The AXA Convention Centre

The event will be held at the Auditorium AXA of the AXA Convention Centre, which is part of an enormous complex located on the main artery of Barcelona that integrates a shopping centre, two hotels, 48.000 m² of offices, a parking lot, two schools, a sport centre and a public park. City communications are excellent and access from Barcelona's Airport and Sants Station is very quick.

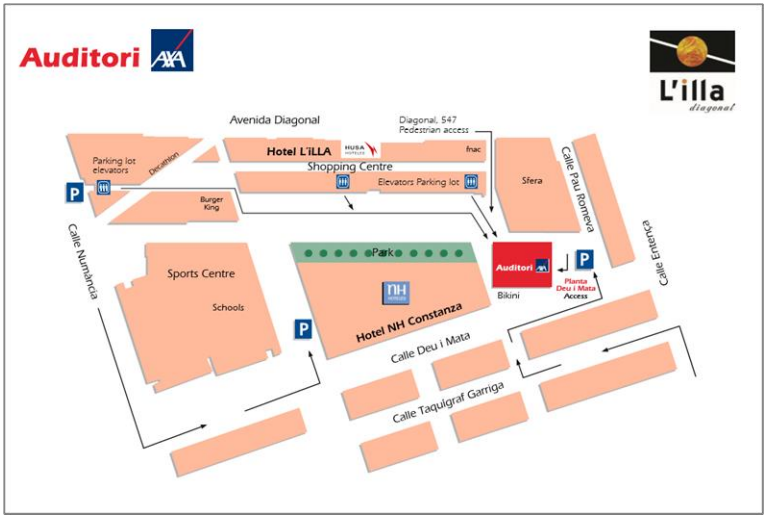
AXA Convention Centre (Source: www.axa.es)

The avant-garde design and construction quality emerge from each and every detail of the building, turning the l'ILLA complex into an emblematic reference of the city.

As a whole, it is more than an auditorium: it is an infrastructure designed to offer quality, flexibility and integral attention through its wide range of services.

How to Reach the Venue

Address: Avinguda Diagonal, 547, 08029 Barcelona, Spain



Venue Location (Source: www.axa.es)

Social Events

Group Visit to Sant Pau Hospital-Art Nouveau Site

Thursday 16 May, 17:15 – 18:30
Price: 18€ *Tickets must be purchased in advance, but you can ask for availability at the Information Desk.*

Central Building and Garden (Source: www.santpaubarcelona.org)

The Modernista Sant Pau Complex was built between 1905 and 1930 and designed by *Lluís Domènech i Montaneras* a garden city for nursing the sick. After being used as a public hospital for a century, its newly refurbished pavilions shine again in all their splendour.

This is Europe's foremost art-nouveau complex and an icon among Barcelona's dazzling array of landmarks which embodies the city's innovative spirit. It was awarded World Heritage status by UNESCO in 1997.

Sant Pau's Pharmacy (Source: www.santpaubarcelona.org)

The exhibition space in the *Sant Salvador Pavilion* takes you on a journey through the history of medicine in Barcelona and one of Europe's oldest healthcare institutions. A visit to this magnificent complex allows you to delve into history, art and the present day, making it a unique experience.

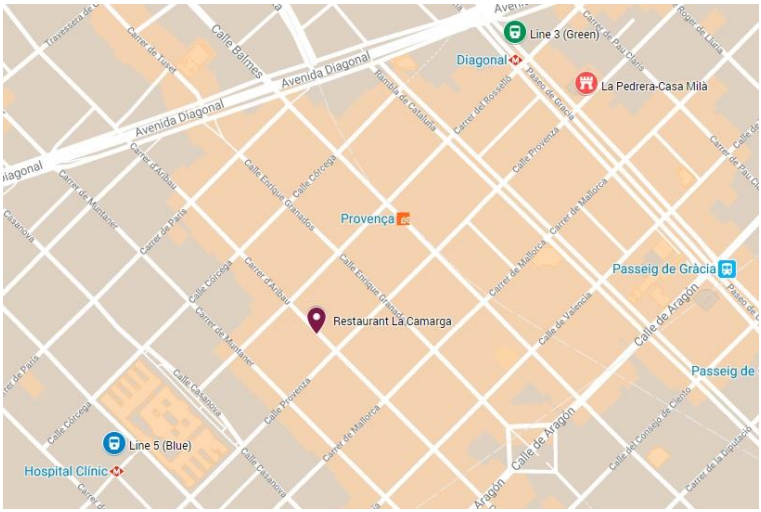
NOTE: The visit includes transfer from the Venue to the Sant Pau Complex, and from there to the Conference Dinner. Attendees will have one **free hour** prior to the Dinner to walk around and discover the *Eixample* area.

Conference Dinner

Thursday 16 May, 20:00
Price: 50€ *Tickets must be purchased in advance, but you can ask for availability at the Information Desk.*

The conference dinner will be held at La Camarga, a cutting-edge restaurant specialized in Mediterranean cuisine with deep gastronomic roots that inspires dishes full of contemporary flavours and textures

La Camarga is located at Carrer d'Aribau, 117, only a few minutes away from *Passeig de Gràcia*, one of the major avenues in Barcelona and one of its most important shopping and business areas, containing several of the city's most celebrated pieces of architecture (such as *La Pedrera* or *Casa Batlló*).For those **not attending** the **Sant Pau** group visit, you can easily reach the restaurant either by taxi, bus or Metro. If you were to choose the second option, the closest metro stations are"Hospital Clinic" on Line 5 (blue), or"Diagonal" on Line 3 (green).



Contact persons during the event

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Emergency Information

All emergencies in Spain: 112 (no area code needed)

Ambulance (Ambulancia) and health emergencies: 061 or 112
Fire brigade (Cuerpo de bomberos): 080 or 112
Spanish National Police (Policía nacional): 091

EUROPEAN FEDERATION FOR MEDICINAL CHEMISTRY

EFMC is an independent association founded in 1970, representing 26 societies from 24 European countries, and more than **7500 medicinal chemists**.

It's main objective is to **advance the science of medicinal chemistry and chemical biology.**

UPCOMING EVENTS

EFMC | Short Course
15th Short Course on Medicinal Chemistry
Small Becomes Big: Fragment-based Drug Discovery
April 28 – May 1, 2019, Oegstgeest, The Netherlands | www.efmcshortcourses.org

EFMC | ACSMEDI
Medicinal Chemistry Frontiers 2019
June 10-13, 2019, Krakow, Poland | www.medchemfrontiers.org

EFMC | ASMC
EFMC International Symposium on Advances in Synthetic and Medicinal Chemistry
Sept 1-5, 2019, Athens, Greece | www.efmc-asmc.org

EFMC-YMCS
6th EFMC Young Medicinal Chemist Symposium
Sept 5-6, 2019, Athens, Greece | www.efmc-ymcs.org

EFMC AWARDS

— The Nauta Pharmacochemistry Award for Medicinal Chemistry and Chemical Biology
— The UCB-Ehrlich Award for Excellence in Medicinal Chemistry
— Prous Institute - Overton and Meyer Award for New Technologies in Drug Discovery

Visit www.efmc.info/awards for more information

EFMC PRIZES

— EFMC Prizes for Young Medicinal Chemists in Industry & Academia

Visit www.efmc.info/prizes for more information

EFMC-YSN: THE YOUNG SCIENTISTS NETWORK

Building a strong network at an early stage in your career is crucial!

The aim of the EFMC-YSN is to inspire, connect and provide opportunities to medicinal chemists and chemical biologists under the age of 35.

Visit www.efmc.info/ysn for more information

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Abstracts

Session 1. Molecules against Cancer

The Targeting of Quadruplex Nucleic Acids in Human Cancers

Stephen Neidle

School of Pharmacy, University College London, London, UK

The overwhelming majority of DNA in the human genome is double-stranded. However, regions comprising several short guanine-tracts are capable of forming higher-order structures, termed quadruplexes. These are not distributed uniformly throughout the genome but are overrepresented in regulatory regions of proliferative genes, in particular those involved in human cancers. Quadruplexes are normally transient and can be read through by polymerases or unwound by helicases. However, they can be stabilised by appropriate quadruplex-selective small molecules and then become effective impediments to transcription, replication or translation.

Crystal-structure analysis has enabled the features of several quadruplex-small molecule complexes to be defined and subsequently used for structure-based optimisation of quadruplex affinity. A small-molecule compound derived in this way will be described, and its pathway to pre-clinical evaluation will be described. it is not specific for a single quadruplex in the genome, but down-regulates a number of key-quadruplex-related genes. This multi-targeting enables the compound to show potent anti-cancer activity in genetically complex pancreatic cancer, which to date has been one of the most intractable of all cancers to treat.

NOTES

FOXM1 Inhibitors: Emergence of a Neglected Binding Force

Seyed Amirhossein Tabatabaei Dakhili, David Javier Pérez Gómez, Keshav Gopal, John Edward Ussher, Carlos Alberto Velázquez Martínez

University of Alberta, Edmonton, Canada

The Forkhead boX M1 (FOXM1) is an essential transcription factor for normal activation of the cell cycle and cell replication. However, increasing evidence suggests that overexpression of this protein correlates with cancer development and poor patient prognosis, which makes FOXM1 a promising drug target in medicinal chemistry. Based on a computer-based molecular modeling protocol reported by our group, we hypothesized that FOXM1 inhibitors bind to the FOXM1 DNA binding domain (DBD) by (i) a pi-sulfur interaction with His287, and (ii) a halogen bonding with Arg297 within the FOXM1 DNA binding domain. To test this hypothesis, we modified the chemical structure of a known "FOXM1 domain inhibitor" (FDI) to synthesize and screen a series of FDI-derivatives. In this regard, we removed or replaced two essential groups in FDI-6, namely (i) the 4-fluorophenyl position and (ii) the heterocyclic sulfur atoms. We determined the inhibitory effects of test molecules on the protein expression of FOXM1 using a triple negative breast cancer cell line (MDA-MB-231), and then we measured their binding affinity to DNA by electromobility shift assay (EMSA). Next, using a site-directed mutagenesis technique, we confirmed specific binding interactions exerted by these molecules. These results validate the role of essential binding interactions (pi-sulfur binding) predicted by computer simulations and provide preliminary evidence to postulate a mechanism of action exerted by "direct" FOXM1 inhibitors.

NOTES

Novel Dual PDK1/AurK-A Inhibitors for Cancer Therapy: Med Chem Evolution and Crystallographic Investigation

Simona Sestito [1], Sara Chiarugi [2], Eleonora Margheritis [2], Massimiliano Runfola [1], Simone Bertini [1], Gianpiero Garau [2], Simona Rapposelli [1]

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The struggle to find novel efficacious pharmacological approaches for cancer treatment has led to the identification of several kinases involved in neoplastic genesis and cell development. Among them, PDK1 (3-phosphoinositide-dependent kinase-1) has a pivotal role in the progression and invasion of tumor masses. Overexpression of PDK1 correlates with an aggressive phenotype and poor prognosis in the brain tumor glioblastoma. Aurora A (Aurk-A) is overexpressed in brain, breast, pancreas, liver, and ovaries cancer. In these malignancies, Aurk-A is involved in multiple mitotic events and pro-oncogenic pathways. The simultaneous inhibition of PDK1 and AurK-A has been proposed as an innovative strategy to overcome glioblastoma resistance and recurrence.

We have developed new molecules that are able to inhibit these two relevant onco-kinases and identified SA16 as a potent dual blocker of both PDK1 (IC50 = 416 nM) and Aurk-A (IC50 = 35 nM). The new pharmacological entity—characterized by a pyridonyl nucleus linked to a 2-oxoindole scaffold through a phenylglycine bridge—was able to disrupt lymphoma, glioblastoma, and glioblastoma-derived stem cell proliferation, prospecting its potential value for therapeutic intervention. With the aim to identify a new class of dual PDK1/Aurk-A inhibitors, we designed and synthesized new compounds in which both the phenylglycine bridge and the substituents on pyridonyl nucleus have been explored. Within the new series of compounds, the SA16-methylated derivative (VI8) showed a more balanced activity profile against the two targets (IC50 = 148 nM for PDK1; IC50 = 69.8 nM for Aurk-A). Crystallographic studies of the kinases in complex with VI8 have finally shown the structural basis for the dual inhibition, which may facilitate the chemical evolution of the promising class of compounds.

NOTES

Development of Thienopyridines as Potent Antiproliferative Agents

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Virtual high throughput screening of a large compound library against the regulatory enzyme phospholipase C (PLC) led to the discovery of the thieno[2,3-*b*]pyridine-2-carboxamides as potential inhibitors. Subsequent biological testing verified the antiproliferative activity of this compound class. Morphology and motility assays, using a number of triple negative breast cancer cell lines, led to the conclusion that PLC is the most probable biomolecular target. Using a combination of computer-aided drug design and synthesis, further analogues have been prepared and tested for their antiproliferative activity, allowing a comprehensive SAR to be developed. Numerous analogues with low nano-molar growth inhibition against various cancers have been prepared. SAR studies suggest that the core structure can be fine-tuned to specific cancers, potentially due to enzyme/isoform specificity. Additionally, mouse xenograft assays showed significant reduction in tumour size after treatment, whilst showing no adverse effects to non-cancerous mice. Here, we report on our recent development of novel thienopyridines and derivatives, expanding the SAR against PLC, and our efforts to prepare potent, soluble, and bioavailable compounds.

NOTES

Tdp1 Inhibition as a Promising Approach to New Anticancer Drugs

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The cytotoxic effects of chemotherapy and radiation that are clinically used to treat malignancies are directly related to their propensity to generate DNA damage. The capacity of cancer cells to recognize DNA damage and initiate DNA repair is a key mechanism for therapeutic resistance to chemotherapy. Therefore, the targeting of DNA repair enzymes can be used as a strategy to potentiate the cytotoxicity of the currently available DNA damaging agents toward cancer cells. PARP1 (poly ADP ribose polymerase 1, the enzyme involved in DNA repair) inhibitors such as olaparib, rucaparib, and niraparib are in clinical use already.

A new and very promising target for antitumor therapy is tyrosyl-DNA phosphodiesterase 1 (Tdp1). It plays a key role in the removal of DNA damage resulting from inhibition of topoisomerase 1 (Topo1) with camptothecin and its clinical derivatives irinotecan and topotecan. Furthermore, Tdp1 is known to be capable of removing the DNA damage induced by other anticancer drugs commonly used in clinical practice.

A set of very potent Tdp1 inhibitors was found by us among natural product derivatives. We designed new inhibitors using targeted modifications of terpenoids, coumarins, usnic acid, and other types of natural products. Moreover, we found that benzopentathiepine derivatives are very effective inhibitors of Tdp1. The ability of the inhibitors used in nontoxic concentrations to enhance the cytotoxicity of camptothecin and topotecan, the established topoisomerase 1 poison, was demonstrated. The significant increase in the antitumor and anti-metastatic effect of topotecan in mice in the presence of Tdp1 inhibitors was shown for the first time. Thus, Tdp1 inhibitors can be considered as a new type of drugs for antitumor therapy.

NOTES

Translating Androgen Receptor Structure into Precision Medicines

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The androgen receptor (AR) plays a crucial role in normal physiology, development, and metabolism as well as in the aetiology and treatment of androgen insensitivity syndromes (AIS), male infertility, neurodegeneration (Kennedy's disease), and prostate cancer (PCa). We have shown that dimerization of AR ligand-binding domain (LBD) is induced by agonists but not by antagonists used in the clinic. The crystal structure of homodimeric, agonist-, and coactivator peptide-bound AR-LBD unveils a large dimerization surface, which harbors over 40 previously unexplained AIS and PCa-associated mutations found in patients [1]. A mutation causing complete AIS located in the protein self-association interface disrupts dimer formation in vivo and has a detrimental effect on the transactivating properties of full-length AR, despite retained hormone-binding capacity. The conservation of essential residues suggests that the AR dimerization mechanism might be shared by other human nuclear receptors involved in several human pathologies. Our work defines AR-LBD homodimerization as an essential step in the proper functioning of this important receptor in the cell. Understanding the detailed molecular aspects of AR dimerization opens novel and unexplored therapeutic avenues to design personalized drugs against castration resistant PCa.

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NOTES

Synthesis and Evaluation of Thymoquinone Analogues as Anti-Ovarian Cancer Agents

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Thymoquinone (TQ), 2-isopropyl-5-methyl-1,4-benzoquinone, a natural product isolated from *Nigella sativa* L., has previously been demonstrated to exhibit antiproliferative activity in vitro against a range of cancers, including ovarian, prostate, colon, breast, pancreatic cancers, leukaemia, and osteosarcoma [1–3]. Recently, TQ has been shown to block substrate recognition by the Polo-Box domain of Polo-like-kinase 1 (Plk1), a mitotic regulator that when overexpressed causes cancer [4]. We describe here the synthesis of a series of analogues of TQ that explore the potential for nitrogen-substitution to this scaffold, or reduction to a hydroquinone scaffold, in increasing the potency of this antiproliferative activity against ovarian cancer cell lines. In addition, alkyl or halogen-substituted analogues were commercially sourced and tested in parallel. Several TQ analogues with improved potency against ovarian cancer cells were found, although this increase is suggested to be moderate. Key aspects of the structure activity relationship that could be further explored are highlighted [5]. In particular, a synthetic aminothymoquinone via substitution of CH of isopropyl group of TQ by a single nitrogen atom showed significant improvement of water solubility, and synergism with two clinically used ovarian cancer drugs: Carboplatin and paclitaxel.

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NOTES

Amphidinolides and Iriomoteolides, Potent Anticancer Macrolides

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Amphidinolides and iriomoteolides are complex macrolides isolated from cultured marine dinoflagellates of the genus *Amphidinium* sp. [1–4] All of them are cytotoxic against several cancer cell lines, especially compounds with larger rings, with activities in the nanomolar range. Some years ago, we started a research program directed towards the synthesis and elucidation of the biological mechanism of action of several members of this family of natural products [5–9]. As part of this research effort, we have completed the total synthesis of some amphidinolides. Work is underway in our laboratories to complete the total synthesis of amphidinolide B_2 and iriomoteolide **2a**.
Amphidinolide K and some of its stereoisomers and analogues were subjected to evaluation of the possible disruption of the α,β-tubulin–microtubule and/or G-actin–F-actin equilibria. Preliminary studies suggest that amphidinolide K behaves as a stabilizer of actin filaments (F-actin) in vitro. The interaction of several of these macrolides with actin has also been studied computationally in our laboratories.

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NOTES

Novel Dipyridothiazines with 1,2,3-Triazole Substituents—Synthesis and Anticancer Activities

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Cancer has now become a global problem and been ranked as the top leading cause of death worldwide after cardiovascular disease, tuberculosis, and malaria combined. Chemotherapy has still been improved in cancer therapy, and the survival has been greatly increased, but there is need to discover and develop new, more potent antitumor agents with better selectivity and reduced side effects. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs with better selectivity and minor or no side effects [1]. Phenothiazines are an important class of heterocyclic compounds with a wide spectrum of biological properties. Recent reports have shown promising anticancer, antiplasmid, antibacterial, anti-inflammatory, and immunosuppressive activities of classical and new phenothiazines [2]. Previously synthesized dipyridothiazine derivatives (1,6-, 1,8-, 2,7-, and 3,6-diazaphenothiazines) were shown to possess interesting antiproliferative, anticancer, antioxidant, and immunosuppressive activity [2–4]. In continuation of our search, we obtained new derivatives of dipyridothiazines with various 1,2,3-triazole substituents in the "click chemistry" 1,3-dipolar cycloaddition. For those compounds, the anticancer action on selected tumor lines (SNB-19, Caco-2, A549, MDA-MB231) was investigated. The compounds exhibited differential inhibitory activities, but some compounds were more active (IC_{50} = 0.02 μg/mL) than reference compound cisplatin. For the most active compounds, the expression of *H3, TP53, CDKN1A, BCL-2,* and *BAX* genes was detected using the RT-QPCR method.

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NOTES

Discovery of a Selective NEK6 Kinase Inhibitor by Virtual Screening

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NIMA-related kinases (Neks) are a conserved serine/threonine protein kinase family related to cell cycle progression and cell division. Among these, NEK6 was shown to be overexpressed in human cancers; its involvement in tumorigenesis has also been demonstrated, thus making NEK6 an emerging attractive target for cancer drug development [1]. The selective inhibitors of NEK6 may therefore become important compounds for identifying novel therapeutic agents. Several natural and synthetic molecules have been reported in literature with inhibitory activity on NEK6, but no potent scaffold has emerged and to date, no inhibitor of NEKs has entered clinical trials for the treatment of cancer. In an effort to identify novel NEK6 inhibitors, we performed a virtual screening study, on a generated homology model of the kinase, adopting both structure- and ligand-based techniques. An in silico study, followed by biochemical screening, led to the identification of (5Z)-2-hydroxy-4-methyl-6-oxo-5-[(5-phenylfuran-2-yl)methylidene]-5,6-dihydropyridine-3-carbonitrile), able to selectively inhibit NEK6, with respect to the homologous NEK7, at micromolar order of magnitude [2,3]. Notably, the compound shows antiproliferative activity against a panel of human cancer cell lines and displays a synergistic effect with cisplatin and paclitaxel in a BRCA2 mutated ovarian cancer cell line, thus supporting a possible use for personalized therapy.

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NOTES

Strategies to Discover p53 Activators and a p73 Activator for Neuroblastoma

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In our quest to discover antitumor agents with novel mechanisms of action, our strategy concerned multiple molecular modifications in a chemical core. As the xanthone molecule can be considered as a privileged structure, particularly in this field of chemotherapics [1], a library of xanthones was built, with several compounds showing promising cell growth inhibitory activity [2]. To disclose the mechanism of action of the most potent derivatives, in silico and in HTS screening approaches were employed [3]. Following this, our group identified LEM1 as αv inhibitor of the p53–MDM2 interaction [4]. The results showed a potent TAp73-dependent cytotoxic activity of LEM2, superior to that of nutlin-3a (a known TAp73 activator), through induction of cell cycle arrest and apoptosis and upregulation of TAp73 target genes, in NBL cells. Additionally, LEM2 sensitized these cells to the effect of doxorubicin or cisplatin. In conclusion, the potent antitumor activity of LEM2 towards primary patient-derived NBL cells, both alone and in combination with conventional chemotherapeutics, may predict promising clinical applications in NBL therapy In tumors with impaired p53 signaling, like neuroblastoma (NBL), one of the most common childhood solid cancers, TAp73-activating agents arise as a promising therapeutic strategy, alternative to p53 activation, to suppress tumor growth and chemoresistance [5]. In the present work, we unveil the discovery of LEM2, a small molecule with a xanthone scaffold, as a new activator of TAp73 with antitumor activity, alone and in combination with conventional chemotherapeutics, in NBL [6].

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NOTES

Organometallic Nucleosides: Synthesis and Biological Evaluation of Substituted Dicobalt Hexacarbonyl Alkynyl Modified 2'-Deoxyuridines

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In continuation of synthetic pursuit of metallo-nucleosides, in particular dicobalt hexacarbonyl 5-alkynyl-2'-deoxyuridines, novel compounds with alkynyl groups were synthesized, starting from 5-iodo-2'-deoxyuridine. Reactions of dicobalt octacarbonyl [$Co_2(CO)_8$] with 2'-deoxy-5-oxopropynyluridines and related compounds gave dicobalt hexacarbonyl nucleoside complexes (83–31%). The growth inhibition of HeLa and K562 cancer cell lines by organometallic nucleosides was examined and compared to that by alkynyl nucleoside precursors. Coordination of the dicobalt carbonyl moiety to the 2'-deoxy-5-alkynyluridines led to a significant increase in its cytotoxic potency. The cobalt compounds' antiproliferative activities against the HeLa cell line and the K562 cell line will be described. Coordination of an acetyl-substituted cobalt nucleoside was expanded using the 1,1-bis(diphenylphosphino)methane (dppm) ligand, which exhibited cytotoxicity at comparable levels. The formation of reactive oxygen species in the presence of cobalt compounds was determined in K562 cells. The results indicate that the mechanism of action for most antiproliferative cobalt compounds may be related to the induction of oxidative stress.

NOTES

Synthesis of Heterocyclic Fused [1,5]naphthyridines by Intramolecular HDA Reactions

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Povarov reaction [1] can be considered as an example of HDA reactions and represents an excellent method for the preparation of nitrogen-containing heterocyclic compounds [2]. When aldimines, derived from aromatic amines and unsaturated functionalized aldehydes, are treated with a Lewis acid, the Povarov reaction takes place intramolecularly [3].

In this work, the synthesis of new families of heterocyclic fused [1,5]naphthyridines is reported. In this way, via an efficient and straightforward intramolecular Povarov reaction catalyzed by boron trifluoride etherate, tetrahydro-6*H*-chromeno[4,3-b][1,5]naphthyridines and tetrahydro-6*H*-quinolino[4,3-b][1,5]naphthyridines are obtained. Dehydrogenation of tetrahydroderivatives with DDQ gives compounds 6*H*-chromeno[4,3-b][1,5]naphthyridine and 6*H*-quinolino[4,3-b][1,5]naphthyridine.

This methodology allows access to novel compounds with biological activity. Based on the success of camptothecin (CPT) and its derivatives as inhibitors of Topoisomerase I (TopI) [4], as well as our results obtained with naphthyridine derivatives [5], we report here that these novel heterocyclic compounds are possible candidates, some of them showing excellent activity as TopI inhibitors. The cytotoxic effect on several cancer and noncancer cell lines was also screened.

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NOTES

Inhibitors of the Inducible Nitric Oxide Synthase as Antiglioma Agents

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Malignant gliomas are highly lethal brain tumors with poor prognosis for patients. The current treatment of glioma consists of maximal surgical resection of the tumor, followed by concurrent chemotherapy (temozolomide, TMZ) and radiation. However, chemotherapy resistance is a major cause of treatment failure.

In general, high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly involved in the malignancy of gliomas as well as in chemoresistance, due to the activation of different signaling mediators. In this context, the dysregulated production of the free radical nitric oxide (NO) by inducible nitric oxide synthase (iNOS) plays a recognized role, and NO inhibition can be considered an emerging therapeutic possibility to treat gliomas [1].

From the development of the acetamidine 1400 W, we recently identified a new small potent molecule, able to selectively inhibit iNOS in rat glioma cells without interacting with the constitutive NOS isoforms [2]. This agent compromises the adaptive responses in glioma cells involved in chemoresistance, enhancing the effects of TMZ [3]. As part of this ongoing project, a new set of acetamidines was synthesized, and the biological results of these molecules will be discussed from a medicinal chemistry viewpoint.

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NOTES

Why Does the Type of Halogen Atom Matter for Radiosensitizing Properties of 5-Substituted 4-thio-2'-deoxyuridines?

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4-thio-5-bromo- and 4-thio-5-iodo-2'-deoxyuridine (BrSdU/ISdU) are well-known photosensitizers that incorporate into a genomic DNA [1]. Such modified DNA becomes sensitive to the photons of ca. 340 nm, which induce potentially lethal damage [1]. For the first time, we recently demonstrated that ISdU also exerts a strong radiosensitizing effect [2]. To our surprise, the viability of MCF-7 cancer cells was only marginally influenced by BrSdU.

In order to explain this unexpected difference between radiosensitizing properties of the two studied derivatives, an experimentally-theoretical study combining stationary and pulse radiolysis with quantum-mechemical calculations was carried out. Thus, the extent of radiolysis as well as the identity and amount of stable products were assayed with the HPLC and LC-MS methods. Furthermore, pulse radiolysis experiments enabled the kinetics and spectra characteristics of transient to be characterized. The experimental results were complemented with density functional theory (DFT) calculations on the thermodynamics and kinetics of dissociative electron attachment (DEA) to the studied compounds and possible secondary reactions.

Our results indicate that the difference observed in the cellular experiments can be attributed to different activation barriers of the DEA process. Indeed, the dissociation of the ISdU anion is a swift process leading to the reactive 4-thio-5-uridyl radical which, if formed in DNA, may lead to serious damage. On the other hand, a larger activation barrier for DEA to BrSdU prevents the release of the bromide anion due to competing processes, such as electron autodetachment, protonation, etc.

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NOTES

In Search of Selectivity: Design, Synthesis and Biological Evaluation of New Classes of HDAC Inhibitors

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Epigenetic regulation of gene expression without changing DNA sequences is a promising strategy in developing therapeutic agents for human diseases, especially cancer. Histone deacetylases (HDACs) are a family of enzymes involved in epigenetic modulation of gene expression by chromatin remodeling, Deacetylation of the lysine side chains of histones by HDAC proteins render DNA transcriptionally inactive, resulting in the inhibition of expression of tumor suppressor genes leading to tumorigenesis and tumor progression. Therefore, inhibiting HDAC enzymes has become an attractive strategy to modulate gene expression as a strategy for developing anticancer drugs. There are currently four FDA-approved cancer drugs in clinical use and many more are in different stages of clinical trials. However, their clinical utility is limited due to undesirable side effects, mainly attributed to their lack of selectivity and the presence of a hydroxamic acid moiety as the metal-binding group. There are eighteen different isoforms of HDACs belonging to four classes that have been identified in humans. A major challenge in HDAC inhibitor development is to make them selective for these HDAC isoforms and classes. We report the design and synthesis of new classes of HDAC inhibitors and the evaluation of their anticancer activity and selectivity.

NOTES

Interaction of Epigenetically-Modified Natural Nucleosides with Membrane Transporters as a Clue to Understand Their Mechanism of Action

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Epigenetically-modified natural nucleosides 5-hydroxymethyl-2'-deoxycytidine (5hmdC) and 5-formyl-2'-deoxycytidine (5fdC) induce cell death in cells expressing cytidine deaminase (CDA) after being converted to their uridine counterparts, 5hmdU and 5fdU, respectively. This finding opened a new avenue in cancer therapy based on selectivity towards tumour cells with high CDA expression [1]. Since 5hmdC and 5fdC and their deaminated metabolites, 5hmdU and 5fdC, are oxidation products of natural pyridimine nucleosides, possible interactions with nucleoside transporters have been evaluated in order to determine their bioavailability. Nucleoside transporters mediate translocation of nucleosides across plasma membrane either in a concentrative, unidirectional, active, energy-costly, and transmembrane sodium gradient coupled manner (hCNTs) or in an equilibrative, bidirectional, and facilitated way (hENTs), encoded by the *SLC28* and *SLC29* gene families, respectively [2]. Experiments have been carried out both in previously generated HEK293 stable cell lines for each transporter, which are routinely used for drug–transporter interaction screening, and in the TFK-1 cholangiocarcinoma cell line with high endogenous CDA activity. 5hmdC and 5fdC have shown interactions with pyrimidine-selective transporters hCNT1 and hCNT3. Afterwards, interaction kinetic constants were determined for each molecule towards hCNT1 and hCNT3. Roughly, these molecules better interact with hCNT3 than hCNT1, and cytidine derivatives are better recognised by both hCNT1 and hCNT3 than 5hmdU. Moreover, the 5-substituent determines the affinity of these molecules towards transporters, formyl being the substituent which most favours the interaction and hydroxymethyl the less favourable one. Interestingly, two synergistic nucleoside–transporter associations have been unveiled in cytotoxicity assays: 5hmdC with hCNT1 and 5fdC with hENT1.

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NOTES

Engineering Affinity, Specificity, and Stability in Protein Therapeutics

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Mesotrypsin is a serine protease that is upregulated with tumor progression and associated with poor prognosis in many human cancers. In cancer models, mesotrypsin promotes tumor growth, invasion, and metastasis, making it an attractive target for therapeutic intervention. To date, no selective inhibitors against mesotrypsin, either natural or synthetic, have been reported. Developing selective inhibitors for mesotrypsin presents special challenges, as mesotrypsin shares high sequence homology and structural similarity with other serine proteases and is resistant to inhibition by many polypeptide inhibitors. The human amyloid β-protein precursor Kunitz protease inhibitor domain (APPI) offers an attractive scaffold for engineering mesotrypsin inhibitors but has the inherent disadvantage of rapid cleavage by the enzyme. In preliminary studies, we have used directed evolution to generate a novel prototype mesotrypsin inhibitor, based on the APPI scaffold, possessing picomolar affinity and improved proteolytic resistance to mesotrypsin. Recently, we were able to identify highly selective novel mesotrypsin inhibitors based on this prototype for clinical translation as imaging and therapeutic agents. Specifically, we used a yeast surface display platform and novel competitive screening strategy to identify selective mesotrypsin antagonists from inhibitor libraries. We have evaluated candidate inhibitors for mesotrypsin selectivity, proteolytic stability, and anticancer efficacy in cell culture models. Finally, we have performed preclinical evaluation of the best candidate as a targeting agent for tumor imaging and as a therapeutic. The proposed strategy is likely to produce mesotrypsin inhibitors of low toxicity and immunogenicity, with substantial translational potential as imaging agents and therapeutics.

NOTES

Synthesis of Polyfluorinated KRN7000 Analogues and Biological Implications

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KRN7000 is a synthetic glycosphingolipid developed as an anticancer drug candidate, which upon association with the CD1d protein activates NKT cells. This event leads to the release of different cytokines, which modulate a TH1 response (antitumoral and antimicrobial functions) or a T_H2 response (against autoimmune diseases). Unfortunately, the simultaneous secretion of both cytokines limits the therapeutic potential of KRN7000, as they can antagonize the biological functions of each type alone. For that reason, the synthesis of new KRN7000 analogues with a more biased T_H1/T_H2 profile is an area of special interest [1].

It has been suggested that T_H1 response is certainly favoured by stabilization of the KRN7000–CD1d–NKT complex [2]. In this regard, it has been recently demonstrated that perfluorinated chains produce stronger interactions with hydrophobic cavities of proteins than its hydrocarbon counterparts [3].

In this communication, we will report the synthesis of set of KRN7000 analogues bearing different perfluoroalkyl chains at the ceramide moiety, with the aim of increasing the stability of the complex to obtain a selective T_H1 response. Biological implications based on binding affinity towards mouse CD1d protein as well as mouse and human iNKT cell stimulation experiments will also be discussed.

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NOTES

Use of a Zebrafish Model to Evaluate Toxicity of Schiff Base Complexes of Copper (II) and Zinc (II) as Possible Antineoplastic Agents

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Cancer continues to be one of the leading causes of death, according to the World Health Organization, and chemotherapy is its principal treatment. Organometallic complexes with copper (II) and zinc (II) with Schiff bases as ligand, capable of interacting with cancer cells' DNA under physiological conditions, may work as good chemotherapy agents because they are less toxic to healthy cells than to cancerous ones. In view of the above, this work focuses on obtaining new Schiff base ligands and their copper and zinc complexes and characterizing them by nuclear magnetic resonance, mass spectrometry, infrared spectroscopy, and other spectroscopic and spectrometric techniques. The objective is to evaluate the toxic activity of the new molecules using the fast proliferating cells of the zebrafish model during development. The toxicity test was performed in zebrafish embryos at 0, 8, and 24 h post fertilization, and a survival and malformation index were registered. Preliminary results show a dose-related effect of the designed Schiff ligands and complexes on the toxicity of the zebrafish embryo and larvae. The survival and malformation index are more severe when exposure occurs during early developmental stages, when cell division is higher due to rapid organization and growth of the new organism. This is a promising result, as the molecules might be cytotoxic to highly proliferating cells, as it occurs in cancer cells. This work represents one of the very few examples that use the zebrafish model to evaluate the cytotoxic activity of Schiff base complexes. Developing the animal model to test the effect of Schiff ligands and their complexes is an important first step to assess the effectiveness of new molecules as antineoplastic agents.

NOTES

Session 2. Targeting Protein Degradation in Drug Discovery

Sag/Rbx2 E3 Ubiquitin Ligase: From Target Validation to Drug Discovery

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SCF (SKP1, Cullins, and F-box proteins) E3 ligase, also known as CRL1 (Cullin-RING ligase-1) as the founding member of CRLs, is the largest family of E3 ubiquitin ligases, consisting of four components: (1) An adaptor protein SKP1, (2) a scaffold protein cullin-1 (CUL1), (3) a substrate-recognizing F-box protein, and (4) a RING protein with two family members, RBX1 or RBX2 (also known as SAG). By promoting ubiquitylation and degradation of many key regulatory proteins, SCF E3s play critical roles in many biological processes, including signal transduction, cell cycle progression, DNA replication, development, and tumorigenesis.SAG/RBX2 is the RING component of CRLs, required for its activity. SAG is overexpressed in a number of human cancers, which is associated with poor survival of patients. We recently found that SAG deletion remarkably suppressed tumorigenesis in the lung, triggered by KrasG12D, and in the prostate, triggered by Pten loss, indicating that SAG is an oncogenic cooperating gene. We have launched a drug discovery project to find small molecule inhibitors that target SAG E3 for anticancer application. More details will be presented.

NOTES

Targeted Protein Degradation with Small Molecules: How PROTACs Work

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Bivalent degrader molecules (also termed PROTACs) target proteins for degradation through recruitment to E3 ligases. PROTACs are a revolutionary new modality class with therapeutic potential. Formation of a ternary complex between the degrader, the ligase, and the target leads to tagging by ubiquitination and proteasomal degradation of the target protein.
In 2015, we disclosed MZ1, a potent degrader made of a ligand we had previously discovered for the E3 ligase von Hippel–Lindau (VHL), and a pan-selective ligand for the BET proteins Brd2, Brd3, and Brd4. We made the unexpected but fascinating observation that MZ1 induces preferential degradation of Brd4 over Brd2 and Brd3—despite engaging BET proteins with the same binary affinity. This demonstrated a now well-established feature of PROTACs: They can achieve a narrower degradation profile in spite of broad target engagement. Our co-crystal structure of a PROTAC ternary complex (VHL:MZ1:Brd4) illuminated the role of cooperative molecular recognition inducing de novo contacts to form a stable ternary. Our work is revealing the structural basis and guiding principles of PROTAC degradation selectivity and mode of action.

NOTES

Expanding the Toolbox of E3 Ligases for Protein Degradation: Targeting the "Undruggable" Fbw7 E3 Ligase

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Proteolysis targeting chimera molecules (PROTACS) are heterobifunctional small molecules designed to induce intracellular protein degradation. The approach works through a validated sub-stoichiometrically catalytic mechanism based on a dual interaction. One tail of the PROTACS binds to a *Protein Of Interest (POI)* while the other tail recruits a specific E3 ligase, forming a ternary complex that allows ubiquitin transfer from the E3 ligase to the POI, which leads to the POI degradation.

The first generation of PROTACS were mainly peptides either for the E3 ligase or for the POI head-ligands; thus, the PROTAC approach had remained largely dormant for over a decade. The recent development and identification of a handful number of specific drug-like molecules targeting E3 ligases has exceptionally improved the perspectives of the PROTAC approach. However, only 5 out of the more than 600 human E3s have reportedly been used to generate PROTACS. The development of small molecules targeting E3 ligases has been rewarded with limited success, partly because modulating their activity and regulation requires targeting protein–protein interactions.

In this talk, I will develop how, following a novel computational, biophysical, and fragment-based approach, we have been able to identify small molecules able to bind to the Fbw7 E3 ligase, which have been considered an *undruggable* target until now.

NOTES

Design, Synthesis, and Biological Evaluation of Bimodal Glycopeptides as Inhibitors of Neurotoxic Protein Aggregation

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Post-translational modifications (PTM) of proteins are becoming the focus of a growing base of research due to their implications in a broad spectrum of neurodegenerative diseases. Various PTMs have been identified to alter their subjects' toxic profiles, playing critical roles in disease aetiology. Regarding Alzheiemer's disease (AD), dysregulated phosphorylation is reported to promote pathogenic processing of the microtubule-associated tau protein. Among PTMs, the enzymatic addition of *N*-acetyl-D-glucosamine (GlcNAc) residues to Ser/Thr residues is reported to deliver protective effects against the pathogenic processing of both of amyloid precursor protein (APP) and tau. Modification of tau with as few as one single *O*-GlcNAc residue inhibits its toxic self-assembly. The modification has the same effect on the assembly of the Parkinson's-associated α-synuclein protein also [1]. A trend is beginning to form, as *O*-GlcNAcylation (*O*-linked GlcNAc modification) affects the processing of the proteins implicated in AD, PD, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) in a similar manner. As such, manipulation of numerous proteins' *O*-GlcNAcylation statuses has been proposed to offer therapeutic routes toward addressing dementia's varied underlying pathologies [2].

Targeting upstream cellular processes sometimes yields mechanism-based toxicity, however, and the enzymes governing *O*-GlcNAc cycling modify thousands of acceptor substrates. We propose that synthetic [3], *O*-GlcNAc-modified peptidomimetics may qualify as useful chemical tools that probe exclusively the effects of GlcNAc-mediated inhibition of protein self-assembly. Moreover, their strong, reversible binding qualifies peptides as model ex vivo imaging agents. Here we have designed, synthesised, and are in the process of evaluating novel bimodal glycopeptides derived from the native α-synuclein sequence for their ability to inhibit wild-type α-synuclein aggregation.

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NOTES

Tracing Potential Covalent Inhibitors of an E3 Ubiquitin Ligase through Target-Focused Modelling

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The Nedd4-1 E3 Ubiquitin ligase has been implicated in multiple disease conditions due its overexpression. Although the Nedd4-1 E3 Ubiquitin ligase is an enzyme that may be targeted either covalently, or non-covalently, there are few studies that demonstrate effective inhibitors of the enzyme. In this work, we aimed to identify covalent inhibitors of Nedd4-1. This task however, proved to be challenging due to the limited available electrophilic moieties in virtual libraries. We therefore opted to divide an existing covalent Nedd4-1 inhibitor in two parts: A non-covalent binding part and a pre-selected α, β-unsaturated ester that forms the covalent linkage with the protein. A non-covalent pharmacophore model was built based on the active site binding investigations followed by validating the covalent conjugation. Thirty compounds were selected and covalently docked into the catalytic site of the Nedd4-1. Multiple filtrations were effected before selecting 5 hits that were later analysed by molecular dynamic simulations to check their stability and explore their binding landscape in complex with the protein. All in all, two inhibitors with optimum overall stability and more stabilising interactions were kept for eventual biological evaluation. Our improved pharmacophore model approach serves as a robust method that will illuminate the screening for novel covalent inhibitor in drug discovery.

NOTES

Structural-Based Virtual Screening to Identify a Class of Small Molecules that Selectively Inhibits Cul-5 Neddylation

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Protein modification by neddylation is catalyzed by a NEDD8-activating enzyme (NAE), a NEDD8-conjugating enzyme (UBE2M or UBE2F), and a NEDD8 ligase, which is required for activation of Cullin–RING ligases (CRLs), which are often abnormally activated in lung cancer cells due to overexpression of few neddylation key enzymes, including NAE, UBE2F, and SAG. A drug discovery effort was made to identify small molecule inhibitors of neddylation for cullin inactivation and tumor suppression. Through structure-based virtual screening of 1.1 million compounds from two small molecule libraries, Chembridge (http://www.chembridge.com/) and SPEC (http://www.specs.net/), we initially identified the top 30 compounds, each with the potential to target two binding pockets (F56 pocket and V30 pocket) in the binding interface of NAEβ-UBE2F or to directly target the enzymatic core of UBE2F (C116), respectively. Our screening read-out is cell-based Western blotting to show inhibition of cullin-5 and accumulation of its substrate NOXA, a pro-apoptotic protein. We found that iV26 worked the best in targeting the V30 pocket, leading to the inhibition of Cul-5 neddylation and marked NOXA accumulation. We then performed three rounds of SAR (structure–activity relationship) optimization of iV26 and identified a leading compound, designated as iV26-9-10-4. Interestingly, unlikethe Ui5 series, which inhibits neddylation of all five cullins, iV26-9-10-4 preferentially inhibited Cul5 neddylation and induced substantial accumulation of NOXA in a time- and dose-dependent manner by significantly prolonging the NOXA protein's half-life. Biologically, iV26-9-10-4 has a strong cell killing ability with an IC50 of 2 μM in multiple lung cancer cells by inducing marked G2/M arrest and apoptosis. The compound is well tolerated in mice after intraperitoneal injection at 50 mg/kg for 2 weeks. In vivo antitumor activity using xenograft models is underway to further evaluate this compound.

NOTES

Session 3. New Avenues in Kinetic Target-Guided Synthesis

How Proteins Catalyze Ligand Formation: Protein-Templated Fragment Ligation Employed in the Validation of Cancer Targets

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Fragment-based drug discovery has been established as a powerful method for the assembly of optimized protein ligands. We employed protein-templated ligations, initially for the site-directed detection of low-affinity fragments and subsequently for the identification of potent fragment combinations that are useful as chemical tools [1–4].

Reversible and irreversible reactions have been employed for ligand construction. Here, we will reflect on the reaction scope of protein-catalyzed ligand formation and consider the thermodynamic and kinetic implications of the reactions. The approach will be demonstrated for the discovery of viral protease inhibitors. Orthosteric inhibitors of the oncogenic protein tyrosine phosphatase SHP2 have been provided and validated in cells and animal models as potent anticancer agents.

Finally, we will show that oncogenic transcription factor STAT5 is able to catalyze Mannich ligation reactions of fragments yielding inhibitors of leukemic cell proliferation. The pH-dependency and protein-specificity of the protein-induced multicomponent reaction was studied and the ligands formed were validated by biophysical studies. They disrupt STAT5–DNA complexes and block phosphorylation of STAT5 and transcription by STAT5 in cells and in animal models inducing apoptosis specifically in STAT5-dependent cells.

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NOTES

Kinetic Target-Guided Synthesis as a Tool for Drug Discovery: Successes, Challenges, and Applications to Metalloproteases

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Target-guided synthesis has emerged as an elegant and efficient lead- and drug-discovery strategy. Using the protein of interest as a vessel to catalyze the formation of its ligands is a rather novel concept, initially used by biochemists. It has gained a real interest in drug discovery in the past ten years.

In a kinetic target-guided synthesis (KTGS) [1], the biological target accelerates an irreversible reaction between a pair of reagents by stabilizing a productive configuration of the ternary complex. If the product is structurally similar to the transition state, its affinity for the protein is significantly improved compared to the affinity of reagents. The “click” chemistry is the most widely used KTGS reaction and was pioneered by Sharpless et al. [2].

After describing the history of the use of KTGS, the successes and challenges of this strategy from both a conceptual and practical point of view will be reviewed with case studies. An analysis of the chemical space of ligands discovered by KTGS will be presented. We will then present our work on the discovery of ligands, from compounds acting on transcriptional receptors to modulators of metalloproteases, among them the insulin degrading enzyme [3]. Finally, we will disclose some of our most recent work on metalloproteases of the M1 family.

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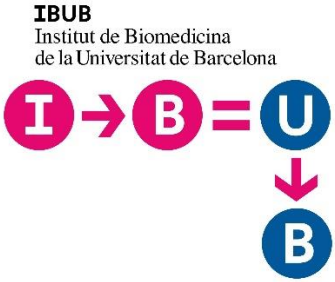

Created in 2006, the strategic plan of the Institute of Biomedicine of the University of Barcelona (IBUB - Drug and Target Discovery) encompasses a variety of actions for the promotion of a research profile oriented towards the discovery of new biological targets and drugs susceptible to become novel generation drugs. For this purpose, there are, in addition to strong biological foundations, well endorsed by groups with extensive experience in cell and animal models suitable for molecular screening, highly recognized groups in the field of computational design of new drugs, and top-level laboratories in organic synthesis. These capabilities are structured in two areas, the Integrative Biology Program and the Drug Discovery Program.

IBUB Director: Dr. Marçal Pastor Anglada, CIBER EHD, and Institut de Recerca Sant Joan de Déu, Barcelona, Spain.

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Abstracts

Session 4: In the Pursuit of Novel Drugs and Molecular Targets

Sponsored by

Targeting Novel Allosteric Sites with Confidence: Methods and Applications

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Allosteric sites create opportunities to act on novel targets when their orthosteric sites are not druggable. However, they also provide increased selectivity and can modulate their targets in unique ways, such as enzymatic activation or change in protein levels, localization or quaternary structure. The discovery of allosteric modulators has traditionally been serendipitous, resulting from random (often phenotypic) screening and subsequent elucidation of their mechanism of action. Computational tools developed in our group for druggability prediction, binding site mapping, and virtual screening have enabled us to identify and tackle novel allosteric sites with confidence. In this talk, I will give an overview of our approach, which combines molecular dynamics with mixed solvents (MDmix) [1–3], molecular docking [4], dynamic undocking (DUck) [5,6] and, finally, experimental screening of a shortlist of candidate molecules in low-throughput assays. I will provide examples of successful discovery of allosteric binders, including non-competitive pharmacological chaperones for the treatment of rare diseases.

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CNS-Selective Estrogen Therapy

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17β-Estradiol (E2), the main human estrogen, has been known to exert multiple actions throughout the body, including in the central nervous system (CNS). In particular, it has been shown that E2 is gender-independently needed for brain and eye health. Lack of E2 due to normal aging and/or pathological processes leads to neurological and psychiatric diseases as well as accelerated neurodegeneration. Current estrogen replacement therapies, however, cannot be used as therapeutic interventions to treat these maladies due to a profound, unwanted hormonal exposure to the rest of the body. In this presentation, we show that the small-molecule bioprecursor prodrug 10β,17β-dihydroxyestra-1,4-dien-3-one (DHED) produces E2 only in the CNS but remains inert in the rest of the body, both upon chronic systemic and topical administrations, thereby avoiding the detrimental side-effects of the hormone, such as stimulation of the uterus and tumor growth. The highly localized production of E2 in the CNS will be shown through a series of bioanalytical assays and efficacy studies using animal models of estrogen-responsive maladies pertaining to the brain and the retina. Owing to DHED's significantly more favorable physicochemical properties than the highly lipophilic parent E2 for transport through biological membranes such as the blood-brain barrier or the cornea, a highly effective E2 therapy can be achieved in rodents upon prodrug administration, which further enhances therapeutic safety. Altogether, our patented DHED approach shows unprecedented selectivity to deliver E2 into the CNS and, thus, promises a high translation value in terms of efficacious and safe treatment against neurodegeneration as well as neurological and psychiatric symptoms arising from estrogen deficiency.

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NOTES

Discovery of Small Molecule Inhibitors That Disrupt the Interaction of Neddylation E1 NAE and E2 Ube2F for Cancer Therapy

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Neddylation, catalyzed by a NEDD8-activating enzyme (NAE), a NEDD8-conjugating enzyme (UBE2M or UBE2F), and a NEDD8 ligase, plays a pivotal role in the activation of cullin RING ligases (CRLs). Two key enzymes, NAE (a heterodimer of NAEα and NAEβ) and UBE2F, were overexpressed in lung cancer. Our work is to discover the small molecules that disrupt NAEb-UBE2F binding, leading to the inactivation of CRLs to suppress cancer cell survival.

Through structure-based virtual screening of 1.1 million compounds from two small molecule libraries, Chembridge (http://www.chembridge.com/) and SPEC (http://www.specs.net/), we identified the top 30 compounds (Ui1, Ui2…Ui90), each with the potential to target the binding interface of NAEb–UBE2F (F56 and V30 pockets) or the enzyme activity center of UBE2F, respectively. The read-out is cell-based Western blotting to show inhibition of cullin-5 and accumulation of its substrate NOXA, a pro-apoptotic protein. We found that Ui5 worked the best in targeting the F56 pocket.

We then performed four rounds of SAR (structure–activity relationship) optimization of Ui5 and identified a leading compound, designated as Ui5-8-11-4-1, that inhibits neddylation of all cullins and caused accumulation of NOXA. Using the CETSA (cellular thermal shifting assay), we found that Ui5-8-11-4-1 directly bound to NAEb, suggesting it is likely an inhibitor of E1. Biologically, the compound suppressed lung cancer cell growth with an IC_{50} of ~5 μM by inducing G2/M arrest and marked autophagy. Pharmacodynamics and in vivo antitumor activity in xenograft models are underway to further evaluate this leading compound.

NOTES

Drugging the Undruggable: Inhibiting MYCN Signalling

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Neuroblastoma is the most common solid malignancy in early childhood, and advanced disease accounts for a disproportionately high mortality when compared with other child cancer types. The *MYCN* oncogene is amplified and overexpressed in 25% of patients with this embryonal childhood cancer. However, the design of MYC inhibitors has been hampered by the lack of globular functional MYC domains or deep protein 'pockets' for drug design. Moreover, MYC inhibitors carry a heightened potential for side-effects due to the dependency of most normal cells on transient MYC expression at entry into the cell cycle.

Enhanced MYCN protein stability is a key component of MYCN oncogenesis and is maintained by multiple feedforward expression loops involving MYCN transactivation target genes, regulated by multiple protein–protein and protein–DNA interactions. Therefore, using a combination of in silico, biochemical, in vitro, and in vivo studies, our multidisciplinary team has developed a program which has characterised protein–protein interactions responsible for MYCN protein stability in Neuroblastoma.

Furthermore, using structure-based drug design methods, we have designed potent and selective small molecules against these protein–protein interactions, which are active in our Neuroblastoma animal models. Here, I present an early stage drug discovery example of this approach.

NOTES

Novel Antimalarial Inhibitors That Specifically Target the Invasion Motor Protein Myosin A in Malaria Parasites

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Malaria remains a devastating disease with nearly half a million deaths per year. The WHO reports a stagnating number of new infections every year without a significant decline, as a result of insufficient access to antimalarials in endemic regions as well as complex resistance mechanisms of the parasites against current treatments. Due to its critical role during the parasite's life cycle, the invasion motor myosin A is a promising target, which has not yet been considered in drug discovery. Myosins appeared to be undruggable since they are ubiquitously expressed and involved in a wide range of cellular processes. In total, the protein superfamily of myosins comprises 35 known subclasses. However, recent studies highlighted the possibility to modulate the myosin motor activity of specific myosin isoforms and classes using small allosteric effector molecules. Exploiting the concept of reversible covalent binding, we show the development of highly potent and specific inhibitors of the key motor myosin A of the glideosome—a sophisticated motor machinery involved in parasite motility and host cell invasion. Combining chemical synthesis with biophysical in vitro analysis confirmed the preferential inhibition of the target protein in the submicromolar range. The developed compounds show significant antiparasitic activities and block efficiently glideosome-associated processes, parasite proliferation, and parasitemia of the malaria parasites. Our findings demonstrate the high potential of our approach using reversible covalent binding to develop new allosteric inhibitors, targeting specifically the key invasion motor as a novel drug target to treat infections caused by malaria parasites.

NOTES

Understanding the Pathophysiology and Searching for Biomarkers for Rare Genetic Developmental Diseases

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Opitz C syndrome (OCS, MIM #211750) is an extremely rare genetic disorder characterized by multiple malformations (e.g., trigonocephaly, congenital heart defects) and variable intellectual and psychomotor delay. In a cohort of 15 families with patients clinically diagnosed as OCS, mutations in 10 different genes were identified as disease-causing by whole exome sequencing (WES). Thus, OCS turned out to be a genetically heterogeneous clinical phenotype. In this project, we aim to functionally characterize two of these causal genes whose de novo mutations were identified in different patients by studying patients' fibroblasts.

De novo heterozygous missense variants in *TRAF7* have been recently associated with CAFDADD syndrome (MIM #618164), whose encoded protein is an E3 ubiquitin ligase involved in different signaling pathways mediated by TNFα. While no significant differences in cell viability were observed between fibroblasts from patients and controls, RNAseq results yielded differentially expressed genes belonging to pathways related to axonal guidance, synapsis, and cardiac hypertrophy.

De novo truncating mutations in *MAGEL2*, a gene included in the Prader–Willi region (15q11–q13), have been associated with SHFYNG syndrome (MIM #615547). MAGEL2 is an essential component of the retromer, involved in endosome to trans-Golgi retrograde transport, and alteration of VPS35 (a MAGEL2 partner) leads to alterations in APP transport from endosomes. In this line, we have found a significant decrease in excreted amyloid $B_{1\text{-}40}$ (Ab) in patients' fibroblasts when compared with controls, making Ab a promising biomarker for SHFYNG syndrome.

Results so far confirm a pathogenic role of these mutations. These data together with future experiments will allow us to determine trustable biomarkers for each disease that could help to better understand its pathophysiology and to monitor the effect of therapeutic drugs.

NOTES

Steroid Sulfatase Inhibition: From Concept to Clinic and Beyond

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**** Molecules 2018 Tu Youyou Award Winner***

Many tumours are hormone-dependent, and estrogens (E1/E2) play a key role in development. Despite aromatase inhibitor (AI) therapy, patients relapse and acquire resistance. Evidence is growing that steroid sulfatase (STS) inhibition will attenuate estrogenic stimulation in hormone-dependent breast cancer (HDBC). E1-3-*O*-sulfamate was the first potent oral, irreversible STS inhibitor, reaching phase II clinical trials. E2-3-*O*-sulfamate is in trials for endometriosis. Superior non-steroidal, non-estrogenic sulfamate-based drugs lead to clinical STX64/Irosustat that is highly orally bioavailable in vivo. Phase I/II clinical trials against locally advanced/metastatic breast cancer showed evidence of stable disease. Trials showed clinical benefit in endometrial cancer and first efficacy both in early breast cancer and in AI combination. A prostate cancer trial has been performed, with further potential elsewhere. Highly potent aryl sulfamate-based STS inhibitors will be discussed around various templates. We investigated inhibition using a soluble bacterial STS, and mechanistic aspects will be reviewed. Dual inhibition of aromatase and STS may address acquired resistance, and high potency inhibitors in vitro and in vivo on both targets will be discussed.

To exploit the aryl sulfamate pharmacophore in hormone-independent settings, we developed STX140, based around endogenous 2-methoxyestradiol. STX140 has potent STS inhibitory activity and a multi-targeted mechanism with striking in vivo anticancer results and in autoimmune inflammatory disease and activity against the hypoxic tumour carbonic anhydrase CAIX. SAR Translation led to non-steroidal systems with a similar activity profile and wide applicability, and steroidal and non-steroidal sulfamates have been co-crystallized with a CAIX mimic. Three agents were co-crystallised with αβ-tubulin, demonstrating the first colchicine site binding of a sulfamate–based ligand and uncovering mechanistic aspects. STS is a promising form of anti-endocrine and attractive clinical target in oncology and the aryl sulfamate pharmacophore a powerful motif for drug design.

NOTES

Inhibitors of Phospholipid-Hydrolyzing Enzymes as Novel Agents against Pulmonary Fibrosis and Diabetes Type-1

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Phospholipid-hydrolyzing enzymes, such as phospholipases A_2 (PLA_2s) and autotaxin (ATX), have attracted medicinal interest because they are involved in the generation of various inflammatory mediators. PLA_2s hydrolyze membrane phospholipids initiating the arachidonic acid cascade, and in particular calcium-independent PLA_2 ($iPLA_2$), have been recognized as a participant in biological processes underlying diabetes development and autoimmune-based disorders. ATX hydrolyzes lysophosphatidylcholine, generating lysophosphatidic acid; both ATX and lysophosphatidic acid are involved in pathological conditions, such as fibrosis and cancer. We have developed several classes of potent PLA_2 inhibitors. In this presentation, we will present new β-lactones as highly potent inhibitors of $iPLA_2$ and a novel class of ATX inhibitors containing the zinc binding functionality of hydroxamic acid. Various novel hydroxamic acids based on either 4-aminophenylacetic acid or non-natural δ-amino acids were synthesized and evaluated [1]. Hydroxamic acids that incorporate a δ-amino acid residue exhibit high in vitro inhibitory potency over ATX. Inhibitor GK442 (IC_{50} 60 nM), based on δ-norleucine, was tested for its efficacy in a mouse model of pulmonary inflammation and fibrosis induced by bleomycin and exhibited promising efficacy [1]. New β-lactones have been synthesized and evaluated for inhibitory potency over $iPLA_2$. A β-lactone substituted at position-3 by a four-carbon chain carrying a phenyl group, and at position-4 by a *n*-propyl group (GK563), was identified as being the most potent $iPLA_2$ inhibitor ever reported (X_I(50) 0.0000021). GK563 was found to reduce β-cell apoptosis induced by pro-inflammatory cytokines, raising the possibility that it can be beneficial in countering autoimmune diseases, such as type-1 diabetes.

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NOTES

How Size Matters: Designing Diverse Fragment Libraries for Novel Drug Discovery

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Fragment-based drug discovery (FBDD) has become a major strategy to derive novel lead candidates for both new and established therapeutic targets, as it promises efficient exploration of chemical space by employing fragment-sized (MW 300) compounds. One of the first challenges in implementing a FBDD approach is the design of a fragment library, and more specifically, the choice of its size and individual members. In order to construct a library that maximises the chances of discovering novel chemical matter, a large number of fragments with sufficient structural diversity are often sought. However, the exact diversity of a certain collection of fragments remains elusive, which hinders direct comparisons among different selections of fragments. Building upon structural fingerprints that are commonly utilised in cheminformatics, we herein introduced quantitative measures for the structural diversity of fragment libraries. Structures of commercially available fragments were retrieved from the ZINC database and filtered by physicochemical properties, after which they were subject to selections with library sizes ranging from 100 to 100,000 compounds. The selected libraries were evaluated and compared quantitatively, resulting in interesting size–diversity relationships. Our results suggested the existence of an optimal size for structural diversity and demonstrated that such quantitative measures can guide the design of diverse fragment libraries under various circumstances.

NOTES

Using a Combination of Fragment-Based and Virtual Screening to Discover Inhibitors for DnaG Primase of Mycobacterium Tuberculosis

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The DNA replication process in Mycobacterium tuberculosis (Mtb) is a promising but underexploited target for the development of novel antibiotics. We have developed an approach to identify inhibitors for Mtb DnaG primase, a key enzyme in the DNA replication machinery of Mtb. For the development process, we have used DNA primase from bacteriophage T7. T7 DNA primase has several structural features that are similar to bacterial (including Mtb) primases, making it an ideal model to study bacterial primases. Using NMR screening, fragment molecules that bind T7 DNA primase were identified and then exploited in virtual filtration to select larger molecules from a virtual library. These molecules were docked to the primase active site using the available T7 primase crystal structure and ranked based on their binding energies to identify the best candidates for functional and structural investigations. Biochemical assays revealed that some of the molecules inhibit T7 primase-dependent DNA replication, and the binding mechanism was delineated via NMR spectroscopy. Importantly, some of the compounds also inhibited the activity of DnaG primase of Mtb, albeit in high concentrations. These molecules were further optimized based on structure–activity relationship (SAR) studies and yielded molecules that could effectively inhibit the activity of isolated Mtb DnaG primase as well as growth of Mycobacterium smegmatis, a nonpathogenic relative of Mycobacterium tuberculosis and an excellent model for studying the effect of antituberculous drugs. Overall, our studies yielded a new class of potential antituberculotics and provided new tools for fragment-based lead discovery.

NOTES

Small Molecules as Potential Inhibitors of the 14-3-3/c-Abl Interaction for the Treatment of CML

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c-Abl is a tyrosine kinase implicated in the regulation of proliferation, adhesion, motility, and cell survival. After phosphorylation on the Thr735 residue, it binds to 14-3-3 proteins that sequester c-Abl into the cytoplasm where it induces proliferation and survival. DNA damage or oxidative stress lead to disruption of the c-Abl/14-3-3 complex, nuclear translocation of the c-Abl protein, and activation of apoptosis. The oncogenic form of c-Abl, Bcr-Abl, is the causative of the development and progression of chronic myeloid leukemia (CML) and is localized exclusively in the cytoplasm, inducing proliferation and inhibiting apoptotic cell death.
The aim of the project is to find small molecules able to disrupt the 14-3-3/c-Abl interaction and thus promote c-Abl nuclear translocation and apoptosis of CML cells.
After an X-ray crystallography screening of an in-house library of small molecules, the coenzyme pyridoxal phosphate (PLP) and the nucleotide inosine monophosphate (IMP) were crystallized and identified as potential modulators of the 14-3-3σ. Analysis of the crystal structures revealed that the phosphate group of both molecules could mimic the phosphorylated motif of the protein partners, hence acting as potential inhibitors.
Biophysical analyses were performed in order to validate the former approach, more specifically with the NMR, FP, and SPR techniques.
After validation of the 14-3-3/c-Abl interaction, the role of PLP and IMP as soft-inhibitors was confirmed. Using IMP and PLP evaluation procedures as reference and positives controls, an FP-based screening of a small library of 48 compounds was carried out. Four compounds (compounds **3**, **8**, **29** and **31**) were found to interact, showing similar binding affinities as the reference molecules and evidencing their behavior as soft inhibitors of the 14-3-3/c-Abl complex. The 6 potential inhibitors were assessed against K562 CML cell lines, and the biological effects on Bcr-Abl expressing cells were promising.

NOTES

Adamantane Analogs: From Anti-Influenza Drugs to Soluble Epoxide Hydrolase Inhibitors for Acute Pancreatitis

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The adamantane scaffold is present in eight approved drugs. It is also featured in several compounds in clinical trials [1]. However, ring-contracted, ring-expanded or heteroanalogs of adamantane have been largely ignored in medicinal chemistry.

Soluble epoxide hydrolase (sEH) is an enzyme involved in the inflammatory pathway, which converts epoxyeicosatrienoic acids (EETs), endogenous chemical mediators derived from arachidonic acid, to their corresponding dihydroxyeicosatrienoic acids. Taking into account that EETs show very potent anti-inflammatory and analgesic properties, it has been proposed that inhibition of sEH may have therapeutic effects in various inflammatory diseases [2].

In this context, several adamantane-based potent sEH inhibitors have been developed, but their low solubility and poor pharmacokinetic profiles have hampered their progress into clinics [3].

Herein, we will present: (a) The design and synthesis of several sEH inhibitors featuring an oxaadamantane moiety in order to improve the potency and pharmacokinetic profiles of previously studied adamantane-derived sEH inhibitors, (b) the screening cascade as well as the in vitro proof of concept (PoC) for selecting a candidate for in vivo studies, and (c) two in vivo PoC in which our candidate reduced inflammation and endoplasmic reticulum stress markers in murine models of cerulein-induced acute pancreatitis (AP).

Of note, AP is a serious and life-threatening inflammatory disease and arises as one of the most common gastrointestinal disorders worldwide. Aside from palliative treatments (analgesics, hydration, antibiotics), there is no standard therapeutic strategy for reducing inflammation in AP.

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NOTES

4,4-Disubstituted *N*-benzylpiperidines: A Novel Class of Fusion Inhibitors of Influenza Virus H1N1 Targeting a New Binding Site in Hemagglutinin

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Influenza epidemics are estimated to result in 3–5 million cases of severe illness and 290.000–650.000 deaths per year. Due to the limited anti-influenza drugs available and the emergence of drug-resistant viral strains, innovative influenza therapeutics directed at new targets and/or with novel mechanisms of action are urgently needed.

In an effort to discover novel anti-influenza agents, several molecules of our diverse in-house library were screened against a panel of different influenza virus strains. Following this approach, we identified structurally novel 4,4-disubstituted *N*-benzylpiperidine compounds as interesting *hit* compounds that display antiviral activity against influenza virus A/H1N1 in the low micromolar range. To investigate the structure–activity relationships, several analogues were easily synthesized, by a one-step Ugi four-component reaction, from commercially available amines, isocyanides, N-substituted piperidones, and a variety of amino acids as carboxylic acid components. The mechanism of action of the most active compounds was examined, taking into account time-of-addition, resistance selection, and functional assays for HA-mediated binding or membrane fusion, suggesting that the compounds inhibit HA-mediated fusion. Mutational and computational simulations proposed an unexplored new cavity near the fusion peptide as the putative binding site for these *N*-benzylpiperidine analogues. Interestingly, a pi-stacking interaction between the *N*-benzylpiperidine moiety with a Phe residue of the fusion peptide represents one of the most relevant ligand–protein interactions. These compounds may be considered useful research tools to explore a new cavity at HA for the design of novel small-molecule fusion influenza inhibitors. The synthesis, biological evaluation, mechanism of action, and computational studies of these *N*-benzylpiperidine derivatives will be reported in this communication.

NOTES

Development of Novel, Potent Phosphatidyl–Choline-Specific Phospholipase C Inhibitors

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The association of the abnormal metabolism of choline-containing phospholipids with various cancers has resulted in the identification of enzymes involved in the phosphocholine cycle as potential therapeutic targets. Phosphatidyl–choline-specific phospholipase C (PC-PLC) plays a pivotal role in this cycle and has been implicated in many signalling processes, demonstrating overexpression in various cancerous tumors, thus providing a viable target for inhibition of cancer cell growth. It has been reported that PC-PLC activation accounts for 20–50% of the intracellular phosphocholine production in ovarian and breast cancer cells of different subtypes.

By virtue of virtual high throughput screening, a number of lead compounds were identified as inhibitors of the PC-PLC enzyme, all of which were identified to bind to key zinc atoms at the active site. To study the PC-PLC inhibitory activity of these compounds and to verify their SAR, an extensive range of novel analogues have been synthesized, varying a number of different structural features.

Analysis of the biological activity of the synthesized analogues showed significantly improved inhibitory activity over the only well-documented PC-PLC inhibitor, D609. These results, as well as the antiproliferative activities of the synthesised compounds, will be reported, providing a comprehensive SAR of these potent compounds and their potential therapeutic applications.

NOTES

Design, Synthesis, and Biological Evaluation of Nucleoside Analogues Acting against *Neisseria gonorrhoeae*

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Antimicrobial resistant *Neisseria gonorrhoeae* (AMR-NG) is a concerning superbug for the global health sector in the 21st century [1]. As per the current scenario, *N. gonorrhoeae* has developed resistance among all the antimicrobial agents for treatment since the mid-1930s [2]. In most countries, the only remaining choice for first-line monotherapy for *N. gonorrhoeae* is extended-spectrum cephalosporins (ESCs), such as cefixime and the more potent ceftriaxone [2,3]. Being the last treatment option for *N. gonorrhoeae*, the emergence of resistance in the case of ceftriaxone treatment has necessitated a dire need for developing novel therapeutic approaches. The suggested long term strategic approach would be exploring novel targets to suppress the resistance of *N. gonorrhoeae* [4]. The enzyme MraY (phosphor-MurNAc-pentapeptide translocase), essential for bacterial cell wall synthesis, fulfills the mentioned requirement, as it has not been explored in a clinical context [5]. Specifically, the enzyme is involved in the lipid-linked cycle of peptidoglycan biosynthesis and is reportedly targeted by naturally-occurring nucleoside antibiotics [5,6]. Because of their completely novel mechanism of action, these MraY-inhibiting candidates are therefore speculated to become a promising, long-term resolution for drug-resistant bacterial pathogens. To explore nucleoside antibiotics as a novel class of antibacterials, we have synthesized simplified analogues of a Muraymycin class of nucleoside antibiotic, having linked it together with three pharmacophores, including uridine, 5′-amino ribose, and the diverse lipophilic side chains. The synthesized analogues of Muraymycin have shown some exciting trends which will be presented in more detail.

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NOTES

Targeting Neuronal Transcription Factor BRN2 in Neuroendocrine Tumors

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No targeted therapies exist against aggressive neuroendocrine tumors; hence, these patients are limited to platinum-based chemotherapy that has not advanced in over three decades. These treatments are merely palliative, and patients generally die within one year. Recently, our group identified the neural transcription factor BRN2 as a major clinically relevant driver of neuroendocrine prostate cancer (NEPC), suggesting targeting BRN2 is a promising strategy to prevent neuroendocrine differentiation or treat NEPC. Moreover, further analysis uncovered that BRN2 expression plays a critical role in other neuroendocrine cancers, such as small-cell lung cancer (SCLC) and Ewing sarcoma (ES). Hence, using the integrated power of a computational drug discovery platform and biological testing, we identified first-in-field inhibitors for BRN2.

First, a homology model of BRN2 protein was generated to perform virtual screening. Based on computational predictions, promising hits were purchased and tested using a series of experiments. Cpd18, a lead BRN2 inhibitor, exhibited profound inhibition of BRN2 in transcriptional assay. Direct binding of Cpd18 to the BRN2 protein was determined by BLI assay. Furthermore, Cpd18 reduced the expression of the neuroendocrine genes SOX2 and NCAM1. Importantly, Cpd18 displayed anti-proliferative activity specifically in the patient-derived $BRN2^{hi}$ NCI-H660 and $42D^{ENZR}$cells while displaying no effect on $BRN2^{low}$ CRPC cells. Moreover, Cpd18 also affected the growth of $BRN2^{hi}$ SCLC and ES cells, selectively. Since Cpd18 showed a promising activity profile, it was subjected to further structure-based lead optimization to enhance the potency and pharmacokinetic properties. Consequently, one of the derivatives Cpd18-94 demonstrated 2μM anti-proliferative activity with an hour of stability in human liver microsomes and reduced the growth of NCI-H660 NEPC tumors significantly in xenograft models.

We anticipate that the developed drug prototypes will lay a foundation for the development of small-molecule therapies capable of combating highly aggressive and lethal form of neuroendocrine tumors.

NOTES

Abstracts

Session 5: Medicinal Chemistry Tales

Tools and Drugs for Purine-Binding Targets—Important Players in Inflammation and Cancer

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Purine and pyrimidine derivatives, such as the nucleotides ATP, ADP, UTP, and UDP, the nucleoside adenosine, and the nucleobase adenine, are important signaling molecules which activate membrane receptors termed P0 (adenine receptors), P1 (adenosine receptors), P2Y, and P2X (nucleotide receptors). P0, P1, and P2Y receptors are G protein-coupled, while P2X receptors are ATP-gated ion channels. There is a metabolic link between P1 and P2 receptor agonists, since the nucleotides ATP and ADP (P2 agonists) are hydrolyzed by various ectonucleotidases, producing the P1 agonist adenosine. While ATP is a danger signal mediating pro-inflammatory effects, adenosine acts as a stop signal inducing anti-inflammatory and immunosuppressive activities.

Despite decades of research, only few drugs have been approved that interact with purine receptors. Recently, new hypes and hopes have been created in the field, mainly due to the gold rush fever in immuno-oncology. Adenosine is one of the strongest immunosuppressant agents of the innate immune system. Cancer cells and tissues can release large amounts of ATP, which is immediately hydrolyzed by ectonucleotidases. These ecto-enzymes, including ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1, CD203a), ectonucleoside diphospho-hydrolase 1 (NTPDase1, CD39), and ecto-5'-nucleotidase (CD73), are upregulated on many cancer cells, leading to the production of adenosine. The cloud of adenosine formed around cancer tissues contributes to immune escape by interacting with adenosine A_{2A} and A_{2B} receptor subtypes (A_{2A}AR, A_{2B}AR) on immune cells. In addition, activation of A_{2B}ARs by adenosine enhances cancer cell proliferation, metastasis, and angiogenesis. Blockade of A_{2A} and A_{2B} adenosine receptors and/or inhibition of adenosine formation by blocking ectonucleotidases are being pursued as novel principles that activate the immune system to defeat cancer.

NOTES

Facing Novel Challenges in Neurodegenerative Diseases Drug Discovery: From Small Molecules to Targeted Therapies

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Neurodegenerative diseases are becoming increasingly prevalent with the aging of the general population. The twentieth century witnessed a significant demographic change in the human population of the industrialized world that is currently followed by a similar shift of life expectancy to upper age ranges in developing countries. The effectiveness of a drug depends on accumulation at the site of action at therapeutic levels. However, challenges such as rapid renal clearance, degradation or non-specific accumulation require drug delivery enabling technologies. Targeted drug delivery is a very promising concept, which still needs improvement for better clinical outcomes. Understanding some of the molecular changes associated to these ubiquitous and widespread diseases has stimulated efforts to develop drugs that specially target key proteins. Protein behavior is scrupulously regulated by a plethora of post-translational modifications (PTMs). It is therefore desirable to develop methods to design rational PTMs to modulate specific protein functions [1–3]. We report different approaches and illustrate their successful implementation in the search for treatment for Parkinson's disease. Computer-assisted design of potent small molecules, together with the development of new carriers, allows a wide range of possibilities for targeted therapies. Knowledge on biochemical processes brings the opportunity to provide treatments that are potentially less toxic and more effective than traditional therapeutic approaches.

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NOTES

Potent and Selective Estrogen Receptor-Beta Agonists which Enhance Memory Consolidation in an Ovariectomized Mouse Model

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Estrogen receptor-beta (ER-beta) is a drug target for memory consolidation in postmenopausal women, while estrogen receptor-alpha (ER-alpha) is linked with the proliferation of certain breast cancer cell lines. While the ligand-binding domains of ER-beta and ER-alpha share less than 60% sequence homology, the ligand-binding pockets of the two subtypes have only minor differences in structure and composition. Nonetheless, these minor differences make the ER-beta binding pocket smaller in volume (282 $Å^3$) compared to the ER-alpha binding pocket (379 $Å^3$). We report a series of potent and selective ER-beta agonists with in vivo efficacy that are A–C estrogens, lacking the B and D rings of the endogenous ligand, estradiol (E2). The most potent and selective A–C analog activates the ER-beta isoform over the ER-alpha isoform by 750-fold, with an EC_{50} of 27 ± 4 nM in cell-based functional assays. The compound exhibits in vivo efficacy for memory consolidation for object placement and object recognition in an ovariectomized mouse model at a 0.5 mg/kg dose by intraperitoneal injection and by oral gavage. This analog does not activate seven other nuclear hormone receptors, does not inhibit CYP1A2 or CYP2D6 and has only weak inhibition of CYP2C9 and CYP3A4, and does not cause significant proliferation of MDF-7 breast cancer cells at doses up to 1000 nM.

NOTES

Towards the Modulation of RNA-Binding proteins: New Compounds Targeting Protein HuR

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RNA-binding proteins (RBPs) have been widely recognized for their pivotal role in the regulation of post-transcriptional processes. Particularly, their complexes with RNA are involved in numerous dysfunctions (i.e., cancer, inflammation, and neurodegeneration) and thus pose the interesting question of whether they could be used as therapeutic targets with clinical relevance. The research efforts of our team in this field have been dedicated to the identification of compounds that are able to modulate protein–RNA interactions, with a special focus on the ELAV (embryonic lethal abnormal vision) protein family.

In the present work, we designed novel HuR ligands based on different scaffolds by applying a structure-based approach. The synthesis of representative compounds of each series was accomplished through multicomponent reactions or equally efficient processes. Afterwards, the structural elucidation of their interaction with HuR was carried out according to an STD (saturation transfer difference)–NMR and in silico combined strategy. The information thus obtained represents the basis to identify compounds that are able to act on the stability of ELAV–RNA complexes, therefore modulating gene expression with an unprecedented mode of action.

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NOTES

Structurally Simple, Readily Available Peptidomimetic 1-Benzyl-5-methyl-4-(n-octylamino)pyrimidin-2(1H)-one Exhibited Efficient Cardioprotection in a Myocardial Ischemia (MI) Mouse Model

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TLR4, a member of the toll-like receptors (TLRs) family, serves as a pattern recognition receptor in the innate immune response to microbial pathogens. TLR4 also responds to endogenous factors produced by different stress stimuli or cell damage and regulates the inflammatory reaction to ischemic injury in the heart. TRIF-related adaptor molecule (TRAM) serves as an adapter that recruits the Toll/interleukin 1 receptor (TIR) domain-containing adapter-inducing IFN-β (TRIF) to activate TLR4, following TRIF-dependent cytokine gene transcription. Based on a known TRAM-derived decoy, a nine-amino acid peptide [1], which corresponds to sequences from the TIR domain, the minimal effective sequence resultant in tetrapeptide was evaluated in cardiomyocytes. Subsequently, a simplified peptidomimetic framework was designed, and ten peptidomimetics of this type were synthesized. One of them, namely 1-benzyl-5-methyl-4-(*n*-octylamino)pyrimidin-2(1*H*)-one (**1**), exhibited high potency and efficacy in vitro. In vitro results and in silico analysis provided evidence for a direct interaction of **1** with the TLR4 complex. Being administered in mice, peptidomimetic **1** was able to block the pathophysiological manifestation of MI, resulting in normalization of CK, LDH, and troponin levels, restoration of the concomitant tissue damage, and a 100% survival rate. Inhibition of TLR4-mediated inflammation in post-ischemic myocardium might be used as a therapeutic approach for developing novel cardioprotective drugs [2].

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NOTES

A Novel Series of Sialic Acid-Based Influenza Virus Inhibitors That Target Influenza Virus Neuraminidase

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Influenza virus continues to be a clinically-significant human pathogen that causes both epidemics and pandemics. Successful inhibition of the viral neuramindase hinders the release of virion progeny from the infected host cell and significantly reduces further virus spread.

We recently described the discovery of highly potent sialosyl sulfonate inhibitors of influenza virus sialidase [1,2]. One of the designed sialosyl a-sulfonate derivatives is a nanomolar inhibitor [2] in a cell-based influenza virus replication assay and has comparable activity to that of anti-influenza drugs zanamivir and the active form of oseltamivir, oseltamivir carboxylate.

Finally, we undertook a protein X-ray crystallographic study that provides atomic-level detail of the binding mode of these sialosyl a-sulfonate derivatives [2].

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NOTES

Repositioning of Dantrolene as a Multitarget Agent for Neurodegenerative Diseases

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Dantrolene is an orphan drug representing the sole therapeutic treatment for malignant hyperthermia, a life-threatening pathology affecting 0.2 in every 10,000 people in the EU. Its biological feature consists of the inhibition of ryanodine receptors, which are responsible for calcium recruitment in striatal muscles and brain. Several literature reports described the neuroprotection exerted by dantrolene in different animal models of AD and, recently, in contrasting neurotoxicity induced by MDMA. Indeed, few of these works investigated its effects at a molecular level, namely on putative targets involved in neuroprotection, and often with contrasting results. Here, we present, for the first time, a comprehensive hypothesis involving, in addition to the well-known calcium antagonism, inhibition of monoamine oxidase B (MAO B) and acetylcholinesterase (AChE) and activation of the carrier of L-acylcarnitine (CAC) as concomitant biological activities responsible for neuroprotection. Dantrolene acts in vitro as a reversible, competitive inhibitor of human MAO B (Ki = 1.0 mM), a reversible, noncompetitive inhibitor of human AChE (Ki = 6.3 mM), and an activator of CAC (EC50 = 28 mM). The potential of repurposing of dantrolene, and the design of dantrolene analogues, possibly endowed with the same activity profile and better pharmacokinetic properties, will be discussed and challenged.

NOTES

Kinetic Characterization of Novel HIV-1 Entry Inhibitors: Discovery of a Relationship between Off-Rate and Potency

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The entry of HIV-1 into permissible cells remains an extremely attractive and underexploited therapeutic intervention point. We have previously demonstrated the ability to extend the chemotypes available for optimization in the entry inhibitor class using computational means. Here, we continue this effort, designing and testing three novel compounds with the ability to inhibit HIV-1 entry. We demonstrate that alteration of the core moiety of these entry inhibitors directly influences the potency of the compounds, despite common proximal and distal groups. Moreover, by establishing for the first time a surface plasmon resonance (SPR)-based interaction assay with soluble recombinant SOSIP Env trimers, we demonstrate that the off-rate (kd) parameter shows the strongest correlation with potency in an antiviral assay. Finally, we establish an underappreciated relationship between the potency of a ligand and its degree of electrostatic complementarity (EC) with its target, the Env complex. These findings not only broaden the chemical space in this inhibitor class, but also establish a rapid and simple assay to evaluate future HIV-1 entry inhibitors.

NOTES

Design and Synthesis of Cysteine Proteases Inhibitors

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Cysteine proteases belonging to the papain superfamily have been recognized as interesting therapeutic targets for the search for new drugs against infectious tropical diseases such as malaria (falcipain), Chagas' disease (curtain), leishmaniasis, and Sleeping sickness (rhodesian), and a number of human pathologies, including cancer, Alzheimer's disease, and osteoporosis (cathepsins). We have reported irreversible inhibitors Dipeptidyl epoxyesters (kinac/KI up to 92,090 M-1/s-1) [1], Dipeptidyl enoates (kinac/KI up to 1,530,000 M-1/s-1) [2,3], Aminoacyl epoxysulfones [4], and also reversible inhibitors Dipeptidyl nitroalkenes (IC_{50} up to 0.44 nM) [5] as inhibitors of parasitic cysteine proteases and cathepsins. Inhibition kinetics and computational studies have been used to study the mode of action of these inhibitors.

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NOTES

Rational Design, Synthesis, and Characterization of Glycoconjugates as Potential Vaccines against Tuberculosis

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Tuberculosis (TB) is the first cause of death from infectious diseases worldwide. Only a single anti-TB vaccine is currently available for clinical use, but its efficacy is debatable. Conjugation of antigenic oligosaccharides, such as lipoarabinomannane (LAM), with antigenic proteins from *Mycobacterium tuberculosis* (MTB), has been recently proposed as a new strategy for developing efficient vaccines. Glycosylation with a(1-6) polymannan has also been investigated in order to improve the biological activity of antigenic proteins. This evidence was the rationale leading to the design, synthesis, and analytical characterization of the *neo*-glycoconjugates herein reported as potential vaccines against TB.

A number of semisynthetic glycoconjugates were prepared, starting from mannose, di-, and tri a(1-6) mannan analogues. Glycans were activated with a thiocyanomethyl group at the anomeric position to address protein glycosylation by a selective reaction with lysines of recombinant Ag85B (rAg85B). Since the immunogenicity of rAg85B was decreased upon glycosylation, the mutants K30 and K282 were designed by replacing lysines involved in the main T-epitope sequences, with an arginine residue (R) to prevent their glycosylation.

The effect of K30R, K282R, and K30R+K282R mutations on the T-cell activity of rAg85B was assessed by an immunological assay. The same test was carried out on the glycosylation products of the mutants. After glycosylation, the K30 mutants completely retained the original T-cell activity, thus resulting in antigenic carriers which might be suitable for the development of glycoconjugate-based vaccines against TB. Moreover, the epitope of rAg85B involved in the interaction with antibodies from different sources was identified by proteolytic affinity-MS. The affinity of rAg85B, mutants, and rAg85B-glycoconjugates for the monoclonal antibody anti-Ag85 was compared by SPR analyses to support the mutagenesis approach.

NOTES

www.3d-qsar.com: A Portal to Build 3-D QSAR Models

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The underlying idea of any field-based 3-D QSAR is that differences in a target propriety, e.g., biological activity, are often closely related to equivalent changes in shapes and intensities of noncovalent calculated interaction surrounding the molecules (also called molecular interaction fields, MIFs). This concept was introduced in 1988 by Cramer et al. with the well-known Comparative molecular field analysis (CoMFA). The procedure to build a MIF-based 3-D QSAR model involves the following steps: Training-set selection, alignment of molecules' conformations, MIF calculation, statistical model definition, model validation, and graphical interpretation. To perform all the steps, any user is asked to install specialized software, either costly or even open source, which require the user to have informatics skills. Here, the very first 3-D QSAR series of web applications is presented by which 3-D QSAR models can be easily built and graphically analyzed. The following web applications are included: (1) Py-MolEdit enables the compilation of the data set through either uploading a list of molecules in any openbabel recognized format or by direct drawing through a java script molecular editor; (2) Py-ConfSerch contains different conformational analysis engines to generate conformational ensembles for each dataset molecules; (3) Py-Align, through automatic molecular alignment software, leads to molecular alignment on up to 16 pre-defined different templates conformations or user selected ones; (4) the Py-CoMFA web application allows the building and validation of the 3-D QSAR model in the same fashion of the original CoMFA software. Different tools are available to inspect the models' results either numerically or graphically, all through a standard web browser and without the need to install any additional program. The portal can be used though any electronic device that can surf the internet, as it has been designed to be fully responsive.

NOTES

Abstracts

Poster Exhibition

1. *Pro-Drug* Silibinin Conjugates: A General Synthetic Strategy and Biological Investigations

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Silibinin is the major component of an extract, known as Silymarin, obtained from the seeds of the milk thistle (*Silybum marianum*). As extensively reported in literature, Silibinin displays multiple biological activities, most of them related to its radical scavenging activity. In the past two decades, in addition to hepatoprotective effects, silibinin has demonstrated remarkable anti-cancer as well as cancer chemopreventive efficacy in pre-clinical cell culture and animal models of several epithelial cancers, including skin, bladder, colon, prostate, and lung [1]. Oral administration of chemotherapeutic agents is the mainstay for the treatment of disease. Sustained release formulations have been crucial for the safe and effective dosing of orally administered drugs [2]. In this frame, the synthesis of a phosphodiester can improve the oral bioavailability of poorly water-soluble chemotherapeutic agents [3].
Here, we reported the synthesis of new silibinin conjugates with 3'-ribonucleotide units [4]. The phosphodiester junction is typically used in *pro-drug* strategies, in which the phosphate group is susceptible to hydrolysis by endogenous phosphatases, allowing the release of the active compound [5]. The new conjugates were prepared in few steps, starting from the 3,5,7,4"-tetra-*O*-acyl-silibinin (and 3,5,7,4"-tetra-*O*-acyl-silybin A and B) and suitable nucleotide phosphoramidites (Uridine and Citydine) and characterized by MS and NMR analyses. We reported the synthesis and characterization of different silibinin and silybin nucleotides conjugates and comparison of their anti-cancer efficacy using different human cancer cells.

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2. The Indole Phytoalexin Derivatives Induced a Significant Inhibition on Src Kinase Activity of Human Cancer Cells

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The Src, a protein kinase, is a family of protein tyrosine kinases (SFKs), and this protein catalyses the phosphorylation of tyrosine. The studies have revealed its key roles in regulating signal transduction from cell surface receptors. The Src kinases act as cytoplasmic signalling machinery through regulating various cellular processes, such as cell growth, differentiation, migration, and survival. The pleiotropic functions of the Src family emphasise the importance of family members which have also been accepted as cellular oncogenes. Indole phytoalexins, which have been identified in various plants, have a structure with indole nucleus with the side chain or a heterocycle containing nitrogen and sulphur atoms. The antiproliferative effects of some phytoalexins have been demonstrated in various cancers. Among the members of phytoalexins, brassinin is known with a dithiocarbamate moiety and S-alkyl piece linked to indole core, and camalexin has an indole structure substituted at position 3 by the 1,3-thiazol-2-yl group. The inhibitory effects of these compounds on cancer cell proliferation have been reported. The aim of this study is to evaluate the effects of compounds on Src kinase activity. Human MCF-7 breast carcinoma and SW480 colorectal carcinoma cells were treated with compounds, and the effects of compounds on Src kinase activity were evaluated by Src-tyrosine kinase assay. The data were also compared with the growth inhibitory potential of compounds. The results have shown that both brassinin and camalexin have significantly inhibited the activity of Src kinase at 10 mM and higher concentrations in MCF-7 and SW480 cell lines ($p < 0.05$). In conclusion, this study is the first to evaluate the role of indole phytoalexins on the Src kinase activity of cancer cells. The data obtained have proven that the indole phytoalexin structure can show anticancer activity as Src mediated. It is thought that existing data will shed light on novel anticancer drug development studies.

3. 3,5-Substituted Oxadiazoles as Catalytic Inhibitors of the Human Topoisomerase IIα Molecular Motor

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Cancer constitutes a group of diseases linked to abnormal cell growth that can potentially spread to other parts of the body and is one of the most common causes of death. A possible approach in its treatment is to halt uncontrolled cell division by inhibiting molecular motors—DNA topoisomerases—that enable topological changes of the DNA molecule during this process [1]. Type II DNA topoisomerases**,** especially topo IIα, are established anticancer targets with inhibitors divided into two groups. Topoisomerase poisons are firmly established in clinical use and act by stabilising the cleavage complex between topo IIα and DNA. However, the induction of the double stranded brakes of the DNA molecule caused by this group is associated with severe side effects, such as cardiotoxicity and induction of secondary malignancies [2]. A second emergent group of catalytic inhibitors attempts to circumvent these challenges and currently embodies four subgroups of mechanistically diverse inhibitors, one of them being compounds that act by prevent binding of the ATP molecule into its binding site [2–4].

Here, we designed, synthesized, and evaluated new derivatives of the 3,5-substituted oxadiazoles that act as catalytic inhibitors of the human topo IIα. Introduction of rigid moieties into the initially available flexible oxadiazoles served to reinforce the interactions with the ATP binding site. Selected compounds also displayed promising in vitro cytotoxicity properties on the investigated MCF-7 cancer cell line. The predicted inhibitor binding geometries were evaluated in classical molecular dynamic simulations, and structure-based dynophore modes were subsequently derived to provide a deeper insight into the molecular recognition of this class of compounds with its macromolecular target.

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4. A General Synthetic Strategy and the Preliminary Biological Screening for New Phosphate-Linked Phenolic Dimers

Maddalena Giordano [1], Valeria Romanucci [1], Roberta Bernini [2], Mariangela Clemente [2], Chapla Agarwal [3], Rajesh Agarwal [3], Giovanni Di Fabio [1], Armando Zarrelli [1]

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Phenolpropanoids are secondary metabolites widely distributed in plants. They are believed to protect human cells against oxidative stress, and in fact, they are able to prevent various diseases associated with it, such as cardiovascular, neurodegenerative, and cancer diseases [1]. Among them, hydroxytyrosol, a small molecule found in olive oil and its by-products, exhibits a powerful antioxidant and anticancer activity [2]. However, polyphenols often present poor pharmacokinetic properties and low bioavailability. An interesting approach towards a new complex would be the combination of two or more polyphenol fragments. This "natural-fragment-based drug-discovery" approach would allow the assembly, also in a combinatorial manner, of libraries based on complex polyphenols in a few steps. This strategy provides us with the possibility of easily modifying both scaffold and decorations and modulating pharmacodynamic and pharmacokinetic properties [3]. In this frame, we planned to join two hydrophenethyl fragments by a phosphodiester bridge to obtain a new class of phosphate-linked phenolic compounds which are potentially more bioactive. The new phenolic dimers were prepared in a few steps and in good yields by an efficient phosphoramidite chemistry combining tyrosol, homovanillic, and hydroxytyrosol phosphoramidite, suitably protected [4]. In particular, we describe the synthesis and the MS and NMR characterization of new phosphate-linked dimers. Finally, a comparison of their anti-cancer efficacy on different human cancer cells was carried out.

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5. A Lipoaminopeptaibol Secreted by Alkalophilic Fungus *Emericellopsis alkalina* Demonstrates a Strong Cytotoxic Effect against Tumor Cell Lines

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Soil fungi are known to produce and secrete antibiotics with a strong antimicrobial effect towards eukaryotic organisms. In many occasions, these compounds belong to peptides that are products of non-ribosomal biosynthesis and are called peptaibols. Many peptaibols are cytotoxic and some of them suppress tumor cell lines much better than normal cells by inducing calcium-mediated apoptosis. The main antimicrobial lipoaminopeptaibol—emericellipsin A—isolated from the fungus *Emericellopsis alkalina* strain VKPM F-1428, which demonstrates promising antifungal activity against different fungal taxons, has been found to exhibit selective cytotoxic activity against HepG2 and Hela cell lines (EC_{50} 2.8 and 0.5 μM, respectively) in MTT assays in vitro. This result corresponds to the standard antitumor antibiotic doxorubicin, which has an EC_{50} value of 440 nM. In a fibroblast toxicity test, emericellipsin A exhibited less cytotoxic activity than doxorubicin (EC_{50} 14 and 0.34 μM, respectively). Therefore, it is less toxic to normal cells than doxirubicin (~40 times), but it yields a more potent cytotoxic effect on tumor cell lines. That is why emericellipsin A can be considered for future more detailed investigations to be an effective antitumor substance.

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6. Selective Activity-Based Probes Targeting Fibroblast Activation Protein (FAP)

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Fibroblast activation protein (FAP) is a type II transmembrane serine protease that belongs to the dipeptidyl peptidase (DPP) family. Although FAP expression is practically non-existent in the majority of healthy adult tissues, it is clearly upregulated in tissue remodeling processes associated with several diseases. These include cancer, atherosclerosis, arthritis, hepatic, and pulmonary fibrosis. This finding has recently sparked intensive research aiming at the clinical implementation of FAP as a diagnostic and/or prognostic biomarker for the aforementioned diseases. Several immunochemical approaches have been reported that can quantify FAP expression. The main drawback of these approaches, however, lies in the fact that some of the commercially available FAP antibodies have been reported to lack specificity. On the other hand, an orthogonal line of research focuses on the quantification of the enzymatically active fraction of FAP, generally relying on peptidic activity-based probes. Developing a selective activity-based assay for FAP has proven to be challenging, owing to the frequently encountered lack of probe selectivity towards prolyl oligopeptidase (PREP, PO). In response, we report a novel series of activity-based FAP probes, based on our potent and selective inhibitor UAMC-1110.

7. Anticancer Activities and Underlying Molecular Mechanisms of Novel Mangostin Glycosides in Human Hepatocellular Carcinoma Hep3B Cells

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and is a leading cause of cancer-related death worldwide. Therefore, exploring effective anticancer agents and their modes of action is essential for the prevention and treatment of HCC. Glycosylation can significantly improve the physicochemical and biological properties of small molecules, such as high solubility, stability increase, and lower toxicity. In this study, for the first time, we evaluated the anticancer activities of mangostin-3-*O*-*β*-D-2-deoxyglucopyranoside (Man-3DG) and mangostin 6-*O*-*β*-D-2-deoxyglucopyranoside (Man-6DG), glycosides of mangostin, against human hepatoma Hep3B cells. Our results demonstrated that Man-3DG and Man-6DG significantly suppressed growth and migration of Hep3B cells. In addition, they induced apoptosis of Hep3B cells by regulating apoptosis-related proteins of mitochondria. Noticeably, Man-3DG and Man-6DG also caused autophage, while cotreatment of the mangostin glycosides with an autophage inhibitor 3MA enhanced the inhibitory effect on Hep3B cell growth, compared to single agent treatment. Moreover, Man-3DG and Man-6DG inhibited the c-Met signaling pathway, which plays a critical role in the pathogenesis of liver cancer. Furthermore, the mangostin glycosides decreased tumor cell-induced angiogenesis in vitro through downregulation of hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF). These findings suggest that Man-3DG and Man-6DG might be promising anticancer agents for HCC treatment with superior pharmacological properties than parent molecule mangostin.

8. Antitumor Activity of Ru-Arene Complexes with 1-Pyrenylphosphines

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Since cisplatin was approved for clinical use in cancer treatment by FDA 50 years ago, the field of chemotherapeutic metal-based drugs has grown enormously [1]. At present, apart from platinum, many other transition metal compounds are being heavily studied. Among them, ruthenium have a special interest for cancer treatment, due to their interesting cytotoxic activity and antimetastatic properties [2]. Recently, we joined this area uncovering a new promising type of compounds of general formula ($Ru(arene)X_2(PPyrR_2)$), in which Pyr stands for the 1-pyrenyl group [3]. These compounds follow some clear structure–activity relationships (SARs).

These encouraging results prompted us to design a second generation of ruthenium complexes based on 1-pyrenylphosphines. Here, we present the full synthesis of the ligands, complexes, and their preliminary cytotoxicity studies against several cancer cell lines. We have found that the activity of the systems is highly dependent on the ligand, the arene, and the halide. With the match combination, very high activities, in the nanomolar range, are obtained in in vitro assays for tumoral cells.

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9. Bis-Pyridazine Derivatives with Anticancer Activity

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Over the last decades, pyridazine derivatives are considered “privileged structures” in medicinal chemistry, with special attention being given to pyridazinones derivatives, which were found to have a large range of biological activities, including anticancer. On the other hand, because of the huge difficulties in cancer treatment, there is an urgent need from the pharmaceutical industry for new anticancer drug candidates. As part of our ongoing efforts in searching for new biologically active entities with anticancer potential, we report here the design, synthesis, structure and in vitro anticancer activity of a new class of pyridazinones derivatives, namely bis-pyridazinones. The structures of the compounds were proven by elemental and spectral analysis: IR, LC-MS, 1H-NMR, 13C-NMR, two-dimensional experiments 2D-COSY, HMQC, and HMBC. A few of the compounds were accepted by the National Cancer Institute (USA) for anticancer screening and were evaluated for their in vitro cytotoxic activity against a panel of 60 human tumor cell lines, representing cancers of the brain, breast, colon, kidney, lung, ovary, prostate, as well as leukemia and melanoma. Three of the tested compounds have proven to be active against non-small cell lung cancer HOP 92 and NCI-H226, CNC cancer SNB-75, renal cancer A498 and UO-31, with a growth inhibition between 50–80 mM. Interestingly, one compound (unsubstituted bis-pyridazinones **I**) has a selective anticancer activity, being active only on non-small cell lung cancer HOP 92, with a growth inhibition of 51.45 mM. SAR correlation has been performed.

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10. Chemically Modified Hemocyanins with Enhanced Antibreast Cancer Activities

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Some cancer cells hyperexpress folate receptors (FR); therefore, folate-derivatized delivery systems are applied for a selective delivery of chemotherapeutics. Recent reports have shown that folate-conjugated immunoglobulin induce an immune response from NK cells against FR-positive melanoma tumor cells [1]. Hemocyanins (Hcs) are respiratory copper-containing glycoproteins that present in the hemolymph of arthropods and mollusks. Numerous studies have shown that Hcs induce a potent Th1-dominant immune response when used as a drug carrier or vaccine adjuvant and nonspecific immunostimulant in cancer or have potential as antineoplastic agents [2].

The aim of this pilot study is to obtain potent and selective anticancer agents based on Hcs isolated from marine snails *Rapana thomasiana* (RtH) and from garden snails *Helix lucorum* (HlH). The proteins were conjugated with folic acid (FO) and ferulic acid (FE) in two-step reactions that involve formation of active N-hydroxysuccinimide folates and ferulates and their subsequent covalent bonding to the Hcs. RtH and HlH conjugates with a different degree of FO and FE substitution were obtained and purified by gel filtration chromatography. Using ATR-FTIR spectroscopy, we observed significant conformational changes in Hc molecules which are ascribed to the chemical modification. Interestingly, the DSC experiments have shown that the thermal stability of all proteins was preserved. RtH-FO, RtH-FE, HlH-FO, and HlH-FE are not cytotoxic to human fibroblasts (BJ cells) even if applied at concentrations as high as 2 mg/mL. The four preparations exhibit an excellent cytotoxic effect to hormone-dependent MCF-7 and hormone-independent triple-negative MDA-MB-231 breast cancer cells, and their selectivity varies within the tested proteins.

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11. Clove Buds Affect MCF-7 Breast Cancer Cell Stress/Survival Pathways and Induce Oxidative Stress, DNA Damage, Cell Cycle Arrest, and Apoptosis

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Breast cancer is reportedly the second most diagnosed cancer worldwide. Several recent epidemiological studies revealed that long-term consumption of plant-derived functional foods is linked with a decreased risk of breast carcinoma. A plethora of studies demonstrated that phenolics, carotenoids, and other plant chemicals display anticancer and several other biological activities, for example antioxidant, anti-inflammatory or immunomodulatory. In this study, the anticancer effects of cloves in the in vitro mammary carcinoma model were assessed.

Flow cytometry, fluorescence microscopy, Western blot and life cell imaging techniques were used to study apoptosis mechanisms and signaling pathways involved after cloves treatment.

We demonstrated that clove buds showed anti- and pro-oxidant properties in a time- and dose-dependent manner, followed by SOD modulation, DNA damage, S-phase cell cycle arrest, and apoptosis induction. Moreover, cloves affected stress/survival signaling pathways such as p38 MAPK, Erk 1/2, Akt, and JNK in the MCF-7 breast carcinoma model.

Our data demonstrated that clove treatment suppressed breast cancer cell proliferation, induced oxidative stress changes, affected several proteins involved in survival pathways, and led to programmed cell death in MCF-7 cells.

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12. Comparative Studies on the Human Serum Albumin Binding of the Investigational EGFR Inhibitor KP2187, Its Hypoxia-Activated Cobalt Complex, and a Series of Clinically Approved Inhibitors

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Binding interactions between human serum albumin and four clinically approved epidermal growth factor receptor (EGFR) inhibitors, Gefitinib, Erlotinib, Afatinib, and Osimertinib, were compared to those of the experimental drug KP2187 and its hypoxia-activated kinetically inert cobalt(III) complex. Since hypoxia is a common feature in many solid tumors, it can be turned into an advantage for selective cancer therapy, as it occurs mainly in the tumor tissue compared to normal tissues. The $[Co(III)(KP2187)(acac)_2]Cl$ complex was confirmed to be activated by reduction to the more labile Co(II) ion in the hypoxic environment of tumors, enabling the selective release of the EGFR inhibitor KP2187 [1]. The protein binding was studied by the combined use of steady-state and time resolved spectrofluorometric and molecular modelling methods. Proton dissociation processes, lipophilicity, and solvent-dependent fluorescence properties of the ligands were investigated as well [2]. The aim of our work was to study and compare the solution chemical properties and albumin binding of the selected compounds, which strongly influence their pharmacokinetic properties. Binding constants calculated on the basis of the various experimental data indicate a weak-to-moderate binding on albumin, with only Osimertinib exhibiting a somewhat higher affinity towards this protein. However, our model calculations performed at physiological blood concentrations of albumin resulted in high (ca. 90%) bound fractions for the inhibitors, highlighting the importance of plasma protein binding.

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13. Design and Evaluation of New Phosphorus Substituted Aziridines as Antiproliferative Agents

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Covalent bond formation has become a safe and effective strategy applied not only by nature but also by the pharmaceutical industry to improve disease pharmacology. In the history of modern medicine, covalent drugs have been broadly used in many therapies for a wide range of human diseases [1]. Many modern drugs hold electrophilic moieties acting as "warheads" that capture the active sites by reacting with endogenous nucleophilic functionalities (e.g., thiols and amines).

In the domain of natural products and related compounds, the aziridine moiety is an illustrative class of warhead, which may react with nucleophilic partners of target enzymes and share a similar reaction mechanism to allow the formation of covalent bonds. Aziridines are also important synthetic targets themselves, since they appear in naturally occurring compounds with applications in medicinal chemistry [2]. Aziridines, as powerful alkylating agents, may act as covalent drugs, having an intrinsic in vivo potency by means of their capability to act as DNA cross-linking agents via nucleophilic ring opening of the three-membered heterocyclic compounds [3].

This work describes an efficient diastereoselective synthetic methodology for the preparation of phosphorus substituted cyanoaziridines though the nucleophilic addition of TMSCN, as a cyanide source, to the C–N double bond of 2*H*-azirine derivatives. The aziridine ring, in these new cyanoaziridines, can be activated by simple *N*-acylation or *N*-tosylation. In addition, the cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549) and human embryonic kidney (HEK293) was screened. *N*–H and *N*-Substituted cyanoaziridines showed excellent activity against the A549 cell line in vitro. Moreover, selectivity towards cancer cell (A549) over (HEK293) and nonmalignant cells (MCR-5) has been observed.

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14. Design, Synthesis, and Anticancer Evaluation of Fused 1,2-Diazine Derivatives

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Cancer is one of the most serious and merciless health problems of humankind, and the number of new cases is expected to increase in the next decades. Despite extensive cancer research in order to find more effective drugs and treatments, cancer chemotherapy is complex and complicated, because of the limited efficacy of drugs, significant levels of toxicity, and lack of selectivity, and the emergence of drug resistance and multidrug resistance make the situation even worse. We report here the design, synthesis, structure, and in vitro anticancer activity of two series of compounds derived from pyridazine and phthalazine. The in vitro anticancer activity was tested on a panel of 60 human tumor cell lines representing cancers of the brain, breast, colon, kidney, lung, ovary, prostate, as well as leukemia and melanoma, to the National Cancer Institute (USA). The test was conducted on a single dose and five dose assay. Notably, from the tested compounds, five of them show very good anticancer activity (superior to Doxorubicin, the NCI standard drug for this type of analysis), with a growth inhibition in the area of nanomolar, between 20–100 nM, on several cancer cell lines: breast cancer MCF7, colon cancer HCT-15, KM12 and SW-620, leukemia K562 and SR, melanoma MDA-MB-435, SK-MEL-5, and UACC-62, and renal cancer A498. SAR correlation in the two series and in between the two series has been performed. One compound has an excellent anticancer activity against breast cancer MCF7 cell, leukemia SR cell, and melanoma MDA-MB-435 cell, in the area of 20 nM.

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15. Design, Synthesis, and Biological Evaluation of 4-Aminoquinazoline Appended-Benzofuran Hybrids as Epidermal Growth Factor Receptor Inhibitors

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Nitrogen-containing heterocycles such as quinazolines and benzofurans have received a great amount of interest in targeted therapies as antitumor drugs [1]. Among quinazoline analogues, 4-aminoquinazolines have established themselves as selective and effective inhibitors of the epidermal growth factor receptor tyrosine kinase (EGFR-TK), which results from competitive binding at the ATP site [2,3]. We envisaged that molecular hybridization based on condensing 7-amino-2-aryl-5-bromobenzofurans with 5-bromo-4-chloroquinazolines in a single molecular platform may provide a more general method for the synthesis of 4-aminobenzofuran hybrids. Retrosynthetic analysis revealed that oximes derived from the 7-acetyl–substituted 2-aryl-5-bromobenzofurans would undergo the Beckmann rearrangement followed by hydrolysis of the acetamide derivatives to afford 7-aminobenzofurans for possible condensation with 4-chloroquinazolines.

The prepared benzofuran appended 4-aminoquinazoline hybrids were evaluated for cytotoxicity in vitro against human lung cancer (A549), epithelial colorectal adenocarcinoma (Caco-2), and hepatocellular carcinoma (C3A) cell lines. Since these tumor cells have been proven to be highly expressed in the cell line of EGFR, we also evaluated representative compounds from each series for potential to inhibit EGFR-TK phosphorylation complemented with molecular docking (in silico) into the ATP binding site of EGFR.

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16. Dibenzylxanthines as Pepck-M Inhibitors for Cancer Therapy

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Phosphoenolpyruvate carboxykinase (PEPCK) is the key enzyme in gluconeogenesis/glyceroneogenesis, which catalyzes the decarboxylation of oxaloacetate to phosphoenolpyruvate. In eukaryotes, there are two isozymes present either in the cytosol (PEPCK-C, PCK1) or the mitochondria (PEPCK-M, PCK2). While PCK1 is found in gluconeogenic tissues and has a very clear metabolic function, PCK2 is expressed in non-gluconeogenic cell types, where its role remains largely unknown. For example, PCK2 is highly expressed in most cancer cells, where it provides a growth advantage to cancer cells in nutrient-poor environments.

A group of C-8 modified 3-alkyl-1,8-dibenzylxanthine derivatives was described as a novel PEPCK-C inhibitor family. We hypothesized that this family of inhibitors could cross-inhibit both PEPCK-C and PEPCK-M due to their nearly identical active center. To determine the validity of our claim, we studied PEPCK-M target engagement using INH2—the most potent compound of the family—and showed similar quantitate inhibitory kinetics to PEPCK-C. Therefore, we validated PEPCK-M as a cancer target using INH2 and compared its efficacy to 3-mercaptopicolinic acid, a classical, low potency PEPCK-C inhibitor.

Treatment of colon and breast carcinoma cell lines with INH2 was shown to inhibit cell proliferation, and it decreased cell survival in poor-nutrient environments. Inhibition of PEPCK-M with INH2 also reduced colony formation in a soft agar model of anchorage-independent growth. Finally, we tested the inhibitor in two subcutaneous xenograft models—SW-480 colon carcinoma and MDA-MB-231 breast carcinoma. Daily dosing with 8.3 mg/kg of INH2 successfully inhibited tumor growth with respect to the vehicle treatment group. There was no weight loss or any sign of apparent toxicity induced by the treatment.

Our results suggest that PEPCK-M is a valid target for cancer treatment with specific inhibitors.

17. Double Modification of Polyether Ionophores: Synthesis and Biological Activity of Novel Salinomycin Derivatives

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Polyether ionophore antibiotics represent a large group of more than 120 lipid-soluble compounds that are widely used in veterinary medicine because of their significant antimicrobial activity. In addition to the industrial use of ionophores, some of them effectively and selectively inhibit properties of different cancer cells and enhance the antitumor effects of chemo- and/or radiotherapy. Salinomycin (SAL) is particularly interesting in this regard, as it shows potent activity against various types of cancer cells, including those that display multidrug resistance, and cancer stem cells. Therefore, a very interesting direction of research is chemical modification of SAL which may lead to obtaining analogs that are characterized by better biological activity and lower toxicity than those of the starting compound.
Within the library of SAL analogs investigated, its C1 and C20 derivatives have shown noteworthy improvements in the biological activity profile. Moreover, our previous studies support the double-modification of SAL as a useful strategy to generate agents with promising biological activity profiles for targeting various types of cancer. For example, it has been proven that the activity of double-modified SAL analogs can surpass commonly used cytostatic drugs in the multidrug resistant cancer cell lines. Here, we report the synthetic access to novel class of C1/C20 doubly modified SAL derivatives, and we present the results of the evaluation of their biological activity.

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18. Ferrocenes as Potential Anticancer Drugs: Determination of the Mechanism of Action

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Chemotherapy is an essential treatment that still plays a vital role in cancer treatment worldwide. The ferrocene derivatives of the general formula [Fe{(η^5-$C_5H_4CH_2$(*p*-C_6H_4)CH_2(N-het)}$_2$] bearing modified six and five membered N-heterocycles were tested in vitro for their cytotoxic properties against ovarian cancer cell lines A2780 and SK-OV-3. These ferrocene complexes displayed cytotoxicity in low micromolar concentrations against both cell lines. To study cellular uptake of particular ferrocenes into tumor cells, we used differential pulse voltammetry and ICP-MS. We confirmed the crucial role of transferrin receptors in the process of intracellular accumulation of these ferrocenes. Interestingly, the rate of intracellular accumulation of particular ferrocenes clearly mirrored the cytotoxicity of these organometallic compounds. Deeper investigation of the mechanism by which ferrocenes kill tumor cells revealed induction of apoptosis associated with significant increase of reactive oxygen species. In conclusion, our screening identified several ferrocene derivatives exerting promising cytostatic activity in vitro. Further investigation led to the identification of the mechanism of action of these potential anticancer agents, which represents an important milestone in preclinical anticancer drug discovery programs.

19. Metformin-Derived Molecules for Glioblastoma Treatment

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Glioblastoma is the most common cerebral tumor in adults. The median survival of glioblastoma patients is 12 months. Metformin is a biguanide used as a standard clinical drug for the treatment of type 2 diabetes. Recently, several studies revealed that the risk of cancer development was significantly reduced for diabetic patients treated with metformin compared to those treated with insulin or sulfonylureas [1]. Even if metformin acts as an antitumoral agent, it is a nontoxic molecule with IC_{50} around 10 mM in cancer cells. In cancer research, naturally occurring phenolic acids are well known to be useful antioxidant agents and allow the inhibition of the migration and adhesion of cancer cells [2]. Moreover, a recent study [3] on nitrones combined with phenolic acids has shown that phenolic acids keep their antioxidant properties even if they are coupled with another molecule. The purpose of this study is to design new molecules combining metformin and a phenolic acid to improve the cytotoxicity on cancer cells.

A series of hybrid molecules was then synthesized. For each molecule, IC_{50} on glioblastoma cell lines (U87 and U251) and on human dermal fibroblasts was tested. After this first screening, the mechanisms through which the best hybrid molecules act on cancer cells were studied and compared with those of metformin. Finally, the study of cytotoxicity on cancer stems cells of glioblastoma, GBM6, and GBM9 revealed that metformin-derived molecules may also restrict the growth of stem cells. As cancer stem cells are one of the causes of tumor resistance [4], metformin hybrid molecules may become a novel therapeutic option to treat glioblastoma.

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20. New 3rd Generation of Casiopeinas Family Compounds with Indomethacin as a Secondary Ligand: Synthesis, Characterization, Antiproliferative Activity and Nanoencapsulation

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The search for new molecules with greater antitumor activity continues, and new forms of administration of chemotherapeutic drugs are the task of this work.
Synthesis and characterization of new seven copper coordination compounds with a general structure of [Cu (NN) (Indo)] NO_3 was carried out, where NN represents bidentate binders of the diimine 1,10-phenanthroline or 2,2'type, -bipyridine with methyl substituents at different positions of the aromatic rings, and Indo represents the indomethacin drug deprotonated.
The study of the redox behavior of the Cu (II)/Cu (I) process shows quasi-reversible systems in which the electronic transfer is carried out slowly. The antiproliferative activity of the compounds was evaluated against a cervical cancer cell line (HeLa). It was observed that the incorporation of indomethacin to Cu-diimine coordination compounds allows to obtain compounds with antitumor activity with values of mean inhibitory concentration (IC 50) in a range between 0.67 and 25.2 μM. The compound [Cu (4,7-dimethyl-phen) (Indo)] NO_3 is one of the most active in the cell line evaluated (0.72 ± 0.10 μM), so it was selected as a model drug to carry out the design of a nanogel sensitive to the stimuli of increased temperature and acid pH, which will allow to control the release of the compound in conditions similar to those found in the tumor zone The release of the compound by the nanogel under different pHs is presented. The system could be useful for the release of the compound in the tumor microenvironment.

21. Photoprotective and Therapeutic Potential in Skin Cancer of Amaryllidaceae Alkaloids

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Skin cancer has evolved as the most common malignant disease, accounting for 4.5% of all new cancer cases. Melanoma, the most aggressive skin cancer type, develops in melanocytes and has high mortality rates due to its biological features and frequent failures of therapeutic alternatives. On the other hand, non-melanoma skin cancers (NMSC), the most common malignant tumors in humans, are developed in keratinocytes of the basal or spinous layer, and increased exposure to ultraviolet (UV) light remains the most important modifiable risk factor. To date, treatment alternatives for melanoma are limited to surgery, in cases of early diagnosis, and a few pharmacological options in inoperable tumors. These limitations for skin cancer management evidence the need to develop therapeutic options for prevention and treatment. The antiproliferative effects of Amaryllidaceae alkaloids have been tested for different types of cancer. However, their activity on skin models is not well-established. Pure alkaloids and alkaloidal fractions characterized by GC-MS of several Amaryllidaceae species from *Crinum, Zephyranthes, Hippeastrum,* and *Eucharis* genera were assayed by their effects on skin cancer. Photoprotective effects of the alkaloids and fractions were determined through cell viability assay, and the quantification of intracellular reactive oxygen species (ROS), and inflammation biomarkers IL-1α, IL-6 and TNF-α, in UVB-stimulated keratinocytes (HaCaT). Cytotoxicity and condensation nuclei were assessed in human metastatic melanoma cells (CRL-3229) to evaluate therapeutic potential, and chemometric techniques were used to analyze data. Most substances enhanced HaCaT proliferation at 5.0 µg/mL. Two alkaloidal fractions and two alkaloids significantly reduced intracellular ROS in UVB-stimulated HaCaT. Additionally, five alkaloids of the tazettine, lycorenine, narciclasine, and haemanthamine types showed antiproliferative activity in melanoma cells. Collectively, these results demonstrate that Amaryllidaceae alkaloids could represent a new option in skin cancer management, acting as photoprotective agents in healthy UVB-exposed keratinocytes and therapeutic agents in melanoma.

22. Recent Advances in Discovery of New Tyrosine Kinase Inhibitors Using Computational Methods

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Tyrosine–protein kinases catalyze chemical reactions that transfer a phosphate group from adenosine triphosphate (ATP) to a tyrosine residue in a protein. Cytoplasmic/non-receptor tyrosine kinases, which act as regulatory proteins, play key roles in cell differentiation, motility, proliferation, and survival. Recent advances have implicated the role of tyrosine kinases in the pathophysiology of cancer. Selective tyrosine kinase inhibitors can block their oncogenic activation in cancer cells and can be applied as a new mode of cancer therapy. Nine Src-family tyrosine kinase have been identified, among which are c-Src and Hck.

Numerous studies have shown evidence of association between c-Src kinases and leukemia. Series of inhibitors (DSA compounds) are based on the central chemical scaffold of imatinib, a drug used for chronic myelogenous leukemia treatment. A series of new 7-chloroquinoline–arylamidine (CQArA) hybrids has been evaluated by quantitative structure–activity relationship (QSAR) analysis in order to signify the importance of structural and chemical attributes for the anticancer activity and propose new analogues with improved activity. The interaction of CQArA hybrids and c-Src in silico has been evaluated by molecular docking based on the binding mode of the DSA inhibitor. It was confirmed that the most active compound binds on the pocket between the small and large lobes of the c-SRC, mostly throughout the hydrogen bonds and van der Waals interactions. Further, an interaction of new 5-arylidenerhodanines and hematopoietic cell tyrosine kinase (Hck) in silico has been evaluated by molecular docking based on the binding mode of quercetin as an inhibitor. Excessive Hck activation is associated with several types of leukemia and enhances cell proliferation. The binding interactions of the most active compound have shown strong hydrogen bonding and van der Waals interactions with the target protein in the catalytic domain of Hck.

Finding new tyrosine kinase inhibitors and their binding modes provides the foundation for the development of new leukemia drugs.

23. Regression of an Epidermoid Carcinomas in Domestic Canine Treated with Casiopeína® IIgly

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Squamous cell carcinoma (SCC) is a malignancy that has no treatment. It develops in the scaly layer of the skin and affects the thoracic, phalangeal, and scrotal limbs, among others (Vet Pathol 2013, 50 (6): 1078). Macroscopically, it presents papillae that can ulcerate and bleed. At histological level, it shows hyperkeratosis, parakeratosis, keratin beads, and cellular pleomorphism (Ginn, 2007, pp. 748–751). On the other hand, Casiopeínas® (Cas) (Ruiz-Azuara, 1992, 1996, 2002, patent US.) have shown antineoplastic activity through mitochondrial apoptosis (Biometals 2017, (1): 43). Casiopeínas represent an important therapeutic alternative for the treatment of SCC.

To evaluate the tumor regression of a canine squamous cell carcinoma by the treatment with Casiopeína IIgly.

After diagnosis of SCC, peripheral blood and urine were collected to perform blood count, urea, creatinine, phosphorus, ALT, AST, and glucose, before and after treatment. The treatment consisted of the administration of 35 mg/m^2 of CasIIgly i.v. for 60 min, in 5% glucose solution. For cytology, two swabs of the lesion were taken every two hours for 6 h. The second and third doses of CasIIgly were administered every 4 h, and two swabs were taken just before the application of the drug. In conjunction with the treatment, a solution of 33.35 mg of CasIIgly dissolved in 33.5 mL of 5% glucose solution was applied directly to the neoplasm, every 12 h for 27 days.

We did not find changes in blood or urine, so CasIIgly did not produce systemic alterations. On the other hand, the neoplastic mass decreased its size, both in diameter and in depth, all suggestive of tumor regression.

The application of CasIIgly following the treatment scheme proposed in this work is effective against CCE in dogs. However, molecular evaluations should still be done to check tumor regression.

24. Rhenium(I) Complexes with Pincer Ligands as a New Class of Potential Antitumor Agents

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Transition metal complexes attract continuous research interest as potential antitumor agents. The most popular compounds are ruthenium, gold, titanium, osmium, iridium, zinc, and palladium complexes, which have already displayed cytotoxic features that are not typical for classical platinum-containing chemotherapeutic agents. Substantially lower attention is drawn to organometallic compounds of rhenium. However, the known examples of cytotoxic organometallic rhenium derivatives with bidentate heterocyclic, organophosphorus, labile alkoxide, and hydroxide ligands render further studies in this field very promising. As for their analogs with multidentate ligands, a literature survey has revealed only a few examples of cytotoxic rhenium complexes, whereas the antitumor activity of cyclometallated derivatives has not been studied at all. At the same time, it is known that the use of pincer-type ligands having specific tridentate monoanionic frameworks, which offer multiple options for directed structural modifications, allows one to finely tune the thermodynamic and kinetic stability of the resulting metal complexes. Therefore, we synthesized and studied the cytotoxic properties of a series of rhenium(I) complexes with tridentate pincer-type ligands based on functionalized carboxamides bearing ancillary donor groups both in the acid and amine components. It was shown that the target complexes can be obtained not only by the conventional solution-based method, but also under solvent-free conditions according to the solid-phase methodology recently developed in our group. The results obtained were used to define the main structure–activity relationships for a principally new class of potential antitumor agents and to choose the most promising compounds for further studies in order to create new pharmaceuticals.

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25. Searching for Molecules against Cancer in the Azores: Plants, Macroalgae, and Synthetic Compounds

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Pursuing the goal of finding active molecules against cancer using various approaches, we focused on natural-based scaffolds in terrestrial plants and in marine macroalgae, taking advantage of the rich biodiversity of the Azores islands. We also focused on molecules obtained by synthesis. In the present work, we report examples of molecules which illustrate these investigations.

Concerning plants such as *Juniperus brevifolia*, an Azorean endemic conifer, we isolated dehydroabietinol, which was active against human tumor cell lines MCF7, A549 and especially against HeLa (IC_{50} = 15.7 µM, with a selectivity index SI = IC_{50}Vero/IC_{50}HeLa of 1.78) [1].

From seaweed *Cystoseira abies-marina*, two new meroditerpenes, cystoazorols A and B, were isolated. Cystoazorol A exhibited the highest growth inhibition against HeLa cells (21.6 and 5.9 µM in lag and log growth phases, respectively), with an SI higher than taxol, the positive control [2].

Finally, we report the antitumor activity of chalcones obtained by chemical synthesis. Among the different compounds synthesized, the best results against A549 cell line were IC_{50} values of 367.4 and 311.4 µM for a chalcone and a flavanone, respectively. Although the activity of these molecules is lower than that of the natural compounds referred above, it should be noted that this activity may be modulated by variating substituent groups.

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26. Syntheses of Hydrazino-and Azo-Sphingosine Derivatives and Their Evaluation as SphK Inhibitors

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Bioactive sphingolipids have been recognized to play important roles in both normal and pathological physiology related to the regulations of cell proliferation, differentiation, survival, trafficking, and cell death. Specifically, overproduction of sphingosine-1-phosphate (S1P) has been associated with a variety of cellular effects that, as a whole, can be described as anti-apoptotic and therefore related with cancer progress. In this respect, sphingosine kinase (SphK), which catalyzes the transfer of phosphate from ATP to sphingosine to generate S1P, has arisen as a therapeutical target in oncologic treatments. Many synthetic sphingolipid analogs have been synthesized with this aim. Thus, FTY720 or CS-0777 are employed as inhibitors of sphingosine kinase in cancer therapies [1]. These compounds, which are characterized by the fact that they contain a quaternary center in the α-position of the amino moiety, could be synthesized through an aziridination reaction. Taking into account the synthesis of N-phthalimido alkynylaziridines in the absence of a metal catalyst reported by Liu [2], we built a family of sphingosine analogues. Syntheses of the starting 2-alkyl substituted α,β-unsaturated esters were accomplished from the corresponding alkyne by a formylation reaction followed by a Wadsworth–Emmons olefination of the aldehyde intermediate. The esters were then submitted to metal-free aziridination followed by a nucleophilic ring-opening process. Final deprotection and further protection of hydrazine moiety and subsequent reduction of ester gave access to a library of analogues. The activity of these analogues as SphK1 and SphK2 inhibitors will also be presented.

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27. Synthesis of Enantiomeric Halolactones with Aromatic Ring, Their Anticancer Activity and Interactions with Biological Membranes

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Studying the effect of chirality on the activity of tested compounds, we synthesized new series of the enantiomeric (ee = 98–100%) β-aryl-δ-halo-γ-lactones with defined configurations of stereogenic centers. The key step of the synthesis was the application of lipase B from *Candida antarctica* to the resolution of racemic (*E*)-4-(2′,5′-dimethylphenyl)but-3-en-2-ol in the process of transesterification. The synthetic pathway included stereospecific Johnson-Claisen rearrangement of resolved alcohols to γ,δ-unsaturated esters followed by their hydrolysis to the corresponding acids and subsequent iodolactonization or bromolactonization. Due to the known configuration of starting allyl alcohols and transfer of chirality during Johnson-Claisen rearrangement, we were able to assign the configuration of all stereogenic centers in the molecules of final lactones.

Obtained halolactones showed high antiproliferative activity *in vitro* against a panel of canine cancer lines: D17, CLBL-1, CLB70, GL-1, and one human cancer line (Jurkat). Their activity was dependent on various structural features: Configuration of stereogenic center, substituents at aromatic ring or a kind of lactone ring, and halogen atom.

In the first step of their biological action, anticancer drugs must penetrate the outer cell membrane to target the intracellular substructures. Therefore, our research was expanded by the studies on interactions of synthesized halolactones with red blood cell membranes. Tested compounds did not induce hemolysis of erythrocytes. The results of fluorescence spectroscopy suggest incorporation of these compounds into hydrophilic part of membrane and no influence on fluidity in the hydrophobic region.

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28. *In silico* Design of E3 Ubiquitin-Protein Ligase NEDD4-1 Inhibitors: An Alternative Approach for Targeting the MAPK Pathway in Cancer Therapy

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Among all the several targets in common use in the antitumor therapies, the pharmacologic inhibition of the MAPK (Mitogen Activated Protein Kinase) pathway represents an efficient therapeutic approach [1]. The MAPK cascade has a pivotal role in the regulation of the cell cycle and proliferation and, within the framework of this pathway, the Sprouty2 protein (Spry2) has emerged as potential target since it negatively modulates the pathway. The intracellular levels of Spry2, whose reduced expression has been associated to tumors [2,3], are regulated by the E3 ubiquitin-protein ligase, NEDD4-1, that ubiquitinates Spry2 for degradation by the proteasome [4].
This research work focuses on a computer aided drug design aimed to identify small molecules able to inhibit NEDD4-1 activity, to restore the intracellular levels of Spry2, which is downregulated in tumors [2,3,5].
A custom computational strategy was employed in order to identify NEDD4-1 inhibitors, based on a multi-step docking-based virtual screening approach and in silico screening of Maybridge and NCI Diversity Set libraries. The 40 highest-ranking molecules from the virtual screening have been submitted to the experimental validation to evaluate their effective ability to inhibit the catalytic activity of NEDD4-1.

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29. Design of New Probes for Oxidized Amino Acids Localization

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Protein carbonyls (PC) are oxidative damage observed in many diseases. Proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often catalysts [1]. A fluorometric and UV-absorption method have been developed to quantify PC in blood and tissues samples by labeling with two hydrazines: 7-hydrazino-4-nitrobenzo-2,1,3-oxadiazole (NBDH) and dinitrophenylhydrazine (DNPH), respectively [2,3]. These methods are based on the selective hydrazone formation between the carbonyls group of oxidized protein to yield a strong fluorescent/UV-absorption adduct that is then quantified.
Here, we will describe our study on NBDH's and DNPH's derivatives to generate new PC-probes bearing an alkyne moiety. In a first step, the hydrazine moiety reacted specifically with protein carbonyls and in a second step, a click reaction was performed between the alkyne moiety and a cleavable resin [4] to yield a PC's enrichment. Theses probes have been explored on oxidized bovine serum albumin (OxBSA) for PC-labeling, and the possible sites of oxidation of isolated labelled PC will be studied by LC-MS and proteomics experiments.

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30. Determination of the Binding Sites of Activators within the Proteasome Structure *

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**** Selected Poster for the Best Poster Award***

The proteasome degrade most of the proteins in eukaryotic cells, thereby controlling the key cellular processes. Impaired degradation mechanisms can lead to accumulation of damaged proteins, resulting in the development of aging processes. Reduced activity of the proteasome also underlies the etiology of some neurodegenerative disorders, such as Alzheimer's or Parkinson's disease. It is believed that low-molecular mass proteasome activators could prevent progression of age-related neurodegenerative disorders.

Blm10 is an activator of the yeast 20S proteasome, which stimulates hydrolysis of peptides and some partially unstructured proteins. The crystal structure of the Blm10-yeast 20S complex revealed that C-terminal residues of Blm10 insert into the pocket between the α5 and α6 subunits of the 20S core particle, and their binding allows to partially open the gate that leads to the catalytic chamber of the proteasome [1].

Blm-pep is a 14-mer peptide, designed based on the sequence of the Blm10 protein, which efficiently stimulates chymotrypsin-like activity of human 20S proteasome. The crystal structure of the complex of Blm-pep and yeast 20S proteasome shows that Blm-pep docks into the same pocket as the C-terminus of Blm10 [2]. So far, we have obtained dozens of analogs of Blm-pep, which stimulate the proteasome peptidases at 10 μM concentration several times. Some of these compounds also are able to enhance the rate of protein substrates degradation in vitro. To determine the place of binding of obtained activators in the structure of human proteasome, we applied different techniques such as cross-linking in combination with mass spectrometry, X-ray crystallography, and molecular modelling. These studies have revealed that there are several sites that are able to bind modulators.

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31. Drugging Challenging E3 Ligases: A Novel Multidisciplinary Approach to Identify Small-Molecules That Bind Fbw7

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During normal cellular homeostasis, proteins are constantly synthesized and destroyed. The most common degradation pathway for proteins is the ubiquitin proteasome system (UPS), a highly regulated signalling cascade that is ultimately responsible for the controlled degradation of a large number of proteins. E3 ligases provide substrate specificity to this system, making them extremely attractive candidates as drug targets. However, the development of small molecules against E3 ligases has led to limited success, in part because modulating their activity and regulation requires targeting protein–protein interactions.

Fbw7 is an important E3 ligase and one of the most commonly deregulated proteins in human cancers. Indeed, 6% of cancers have mutations in the fbw7 gene. On one hand, the loss of activity of the mutated Fbw7 results in a loss of its tumor suppressor function and an upregulation of the natural and oncogenic substrate proteins: c-Myc, cyclin-E, Notch, etc. On the other hand, the inhibition of Fbw7 has been proposed as an approach to sensitize cancer stem cells to chemotherapies. However, so far, no potent small molecules directly targeting Fbw7 have been reported. In this project, using a novel multidisciplinary approach, we aim to identify small molecules that target Fbw7 to disentangle the more convenient pharmacological strategy to manipulate it.

To identify *ligandable* allosteric sites in the Fbw7-Skp1 surface, we applied MDmix simulations. Docking-based virtual screening applying the Duck filter was performed to find potential *hits*. These potential *hits* were tested by surface plasmon resonance and confirmed by STD-NMR. Following this workflow, we were able to identify molecules that target Fbw7 in the one digit micromolar range. In parallel, a fragment-based screening was performed and several fragments were also identified. Work is ongoing to obtain structural information that will confirm the binding site and binding mode of these *hits*.

32. 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. Six percent of cancers have mutations in the *fbw7* gene. On one hand, the loss of activity of the mutated Fbw7 results in a loss of its tumor suppressor function and an upregulation of the natural and oncogenic substrate proteins, such as c-Myc, cyclin-E, and Notch. On the other hand, the inhibition of Fbw7 has been proposed as an approach to sensitize cancer stem cells to chemotherapies. Given the key role of Fbw7 in tumorgenesis, 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.

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. 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. The crystal structure of fragments binding to the protein will not only show the key points for the interaction, but it can also provide the starting point for a rational design to grow the molecules in order to improve their affinity and specificity.

33. Synthesis and Bioactivity Studies of Some Naphthoquinone Derivatives as Potential Proteasome Inhibitors

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The ubiquitine–proteasome pathway (UPP) plays a major role in protein degradation in eukaryotic cells. It has been shown that this pathway is involved in many physiologically critical cellular processes. As the main component of the UPP, the 26S proteasome unit is responsible for the degradation of polyubiquitinated proteins and has multicatalytic proteinase activities. Increased levels of this enzyme have been implicated in many disorders, including inflammation, neurodegenerative, immune diseases, and cancer. Thus, the development of proteasome inhibitors has emerged as an attractive target for the treatment of these diseases, especially cancer [1]. Bortezomib, Ixazomib, and Carfilzomib have been approved by the US Food and Drug Administration (FDA) for the treatment of multiple myeloma. Despite the remarkable success of these inhibitors in the clinic, they have several shortcomings. Therefore, there is still a need to develop new and selective proteasome inhibitors [2]. The compound named PI-083, bearing naphthoquinone group, has recently been reported as a proteasome inhibitor. It has been shown that PI-083 has a broader antitumor activity and is more selective against cancer cells compared to Bortezomib [3]. On the basis of these findings, using a PI-083 lead compound, we designed and synthesized some sulfonamide and carboxamide derivatives bearing naphthoquinone pharmacophoric group as potential proteasome inhibitors and then evaluated their cytotoxic and proteasome inhibitory activities on a human breast cancer cell line (MCF-7). According to the biological activity results, the compounds showed cytotoxic activity at various ratios, and the sulfonamide derivative bearing 2-chloro-3-pyridyl group on amide nitrogen exhibited significant proteasome chymotripsin-like activity inhibition compared to the lead compound PI-083.

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34. Exploring Target-Guided Multicomponent Reactions: Synthesis of Druglike Inhibitors via the Groebke–Blackburn–Bienaymé Reaction Using Urokinase (uPA) as a Model Target *

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**** Selected Poster for the Best Poster Award***

Target guided synthesis (TGS) has emerged as a promising strategy in drug discovery. It relies on direct assistance of a drug target, which serves as a physical template that selects ligand building blocks demonstrating target affinity. The selected building blocks are then assembled into finalized ligands based on complementary chemical reactivity and close proximity in the ligand binding site. On the other hand, building blocks that lack target affinity are not brought to reaction, so that the corresponding ligand constructs are not formed. This effect is known as “selective ligand amplification”. In this way, TGS allows to combine several aspects of drug design, including synthesis and potency determination, in a single step.
While reported examples of TGS generally involve two-component reactions, we are particularly interested in introducing this methodology to multicomponent reactions (MCRs). Given the ability of MCRs to deliver druglike, highly diversified scaffolds, the latter can be expected to greatly expand the scope of TGS. In this study, we selected the Groebke–Blackburn–Bienaymé (GBB) three-component reaction for inhibitor discovery for oncology target urokinase (uPA). Previously, we reported a series of imidazopyridine inhibitors of uPA, which served as reference compounds in our "on-target" work [1]. The feasibility of TGS of uPA inhibitors via the GBB reaction was assessed by molecular docking. The latter proved the compatibility of the target’s active site with the reaction process. Next, a detailed investigation of multiple experimental parameters was performed. It included selection of appropriate reaction medium, enzyme/reactant concentrations, as well as a suitable method for analysis and quantification of the experimental results. Additionally, different strategies to stabilize imines, key intermediates in the GBB reaction, were addressed here.

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35. Immobilized Enzyme Reactors Based on Nucleoside Phosphorylases (NPs) and Nucleoside 2'-Deoxyribosyltransferases (NDTs) for the In-Flow Synthesis of Nucleoside Analogues

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Enzymes are increasingly used as biocatalysts for the production of pharmaceutical products and fine chemicals [1,2]. A well-known strategy to increase catalyst stability and to allow its use for multiple cycles is enzyme immobilization. Moreover, when flow systems are employed for biocatalyzed reactions, the process can benefit from accurate control of the reaction parameters, improved mass transfer, and reduction of the possible substrate/product inhibition of the enzyme [3].

Immobilized enzyme reactors (IMERs) can be successfully applied in drug discovery, for the rapid preparation of small amounts of different compounds. In addition, single or multi-enzyme IMERs might be connected to different separation and detection systems.

In this work, two analytical IMERs were developed as prototypes for biosynthetic purposes, and their performances in the in-flow synthesis of nucleoside analogues of pharmaceutical interest were evaluated. Two classes of enzymes were tested: Nucleoside phosphorylases (NPs) and nucleoside 2'-deoxyribosyltransferases (NDTs).

The NP-based bioreactor was prepared by co-immobilizing uridine phosphorylase from *Clostridium perfringens* (*Cp*UP) and a purine nucleoside phosphorylase from *Aeromonas hydrophila* (*Ah*PNP) on an aminopropyl silica column [4], while the second IMER was obtained by the covalent immobilization of nucleoside 2'-deoxyribosyltransferase from *Lactobacillus reuteri* (*Lr*NDT) on an epoxy silica column. As the chromatographic support, a monolithic material (Chromolith® columns) was selected due to its low operative back-pressure and fast mass transfer.

The in-flow synthesis of 5-iodo-2'-deoxyuridine, 5-fluoro-2'-deoxyuridine, and 2',3'-dideoxyinosine was then performed by exploiting both *Cp*UP/*Ah*PNP and *Lr*NDT-IMERs. Reaction monitoring and conversions were assessed by a reverse phase liquid chromatography system coupled to UV detection. Despite the lower amount of immobilized enzyme, *Lr*NDT-IMER allowed to reach higher conversions in shorter times for the investigated reactions.

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36. New Methodology to Access 1,5-Disubstituted 1,2,3-Triazoles

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1,2,3-triazole is a well-known scaffold that is commonly encountered in bioactive compounds, such as antimicrobial, antiviral, and anticonvulsant drugs. I, the structural properties of 1,2,3-triazole enable it to mimic different chemical functions, such as amides, esters or some heterocycles, justifying its broad use as a bioisostere to synthesize novel bioactive molecules. Moreover, they are remarkably stable to metabolic transformations. In the 2000s, the use of 1,2,3-triazole in medicinal chemistry was intensified due to the copper catalyzed azide–alkyne cycloaddition (CuAAc) methodologies reported simultaneously by Sharpless et al. and Meldal et al., affording the regioselective formation of 1,4-disubstituted 1,2,3-triazoles. By contrast, the regioselective formation of the 1,5-disubstituted regioisomer is less described. Krasiński et al. developed a methodology from an azide and a magnesium acetylide. Hlasta et al. described a method from an azide and an alkyne substituted by a trimethylsilyl group. However, these two methodologies are quite difficult to set up. In recent years, catalytic synthetic pathways have appeared using either ruthenium (RuAAC) or nickel (NiAAC) catalysts. However, despite all these efforts, the scope of substrates is quite limited, preventing the formation of fully functionalized molecules of interest. In order to improve the access of 1,5-disubstituted 1,2,3-triazoles, we have developed a new methodology. The conditions screening and the scope and limitations will be presented.

37. (2-Imidazolin-4-yl)phosphonates: Green Chemistry and Biology Walk Together

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2-Imidazoline-containing compounds constitute a valuable class of agents that modulate a_2-adrenergic receptors and often show a high affinity for imidazoline I_2-receptors (I_2-IR). Moreover, 2-imidazolines are an important class of heterocyclic scaffolds found in natural product chemistry, coordination chemistry, and homogeneous catalysis. To meet the demand for 2-imidazoline-containing compounds, different synthetic approximations were developed. In this work, we describe an efficient and user-friendly synthetic process involving the combination of isocyanide-based multicomponent reaction and microwave heating without the need of anhydrous atmosphere or additional solvents that generates unprecedented (2-imidazolin-4-yl)phosphonates [1].

We assessed the pharmacological profile and selectivity of the prepared compounds upon I_2-IR. Owing to the outstanding high I_2-IR affinity of one of the prepared compounds and high selectivity devoid to the a_2-adrenoceptor of other compounds, markedly better than any described I_2-IR ligand to date, (2-imidazolin-4-yl)phosphonates might be considered as a suitable scaffold for designing novel I_2-IR ligands [2]. In addition, we demonstrated the effectiveness of two of the new I_2-IR ligands in an in vivo female model for cognitive decline (SAMP8), and we analyzed the pathological biomarkers for neurodegeneration. This study is the first experimental evidence that demonstrates the possibility of using this receptor as a target for cognitive impairment [3].

In this work, green chemistry to access an unprecedented scaffold and promising pharmacological results in the neurodegeneration field walked together.

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38. *In Silico* Design of Bacterial *N*-acetylglucosaminidase Inhibitors with Potential Antibacterial Activity

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Staphylococcus aureus is a widespread gram-positive pathogen in humans and animals. Autolysin E (AtlE) is an enzyme from *S. aureus* which belongs to the glycoside hydrolase 73 family. It catalyzes the hydrolysis of the β-1,4-glycosidic bond between the *N*-acetylglucosamine and *N*-acetylmuramic acid units of bacterial peptidoglycan [1]. Autolysins play an important role in biofilm formation, cell growth, and reproduction of bacteria, and they are involved in the separation of the daughter and mother cells during vegetative growth and cell division [1,2]. Cells without *N*-acetylglucosaminidases have morphological abnormalities as a result of their reduced ability to increase size following cell division and to expand into the mature morphology [3,4]. We have applied in silico methods for the discovery of novel AtlE inhibitors. According to the crystal structures of the AtlE–ligand complexes (PDB ID: 4PI7, 4PI9) [1], structure-based virtual screening was employed for hit identification. Based on the results of virtual screening with Gold and Discovery Studio software, we chose the ligands for the quantitative analysis of their binding interactions to AtlE. The experimental tests with surface plasmon resonance technique (SPR) are currently in progress. Identified hit compounds that interact with AtlE will represent valuable starting points for further development of autolysin inhibitors acting via a novel mechanism leading toward novel antibacterials.

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39. *Quorum Sensing* in Cyanobacteria and the Origin of Blooms. Lessons for Human Pharmacology

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Quorum Sensing (*QS*) is a bacterial communication system involved in pathogenicity, virulence, and resistance. On the other hand, the presence of cyanobacteria in water bodies has important implications for public health, because the production of toxins causes death at lower concentrations. The biochemical mechanism of cyanobacterial blooms is unclear, although some causative agents have been proposed, such as high levels of N and P, high temperature, and radiation, among others. Autoinducer lactones of *QS* have not been detected without doubt in cyanobacteria but could explain the formation of blooms in a more rational way.

In his paper, we reported the evaluation of nine homoserine lactones on the growth, the production of cyanotoxins, and colony formation in the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*. *N*-dodecanoyl-L-homoserine lactone was the main inducer of the growth of *M. aeruginosa*, and N-Butyryl-DL-homoserine lactone for *C. raciborskii* (177% and 260%, respectively). In addition, *N*-octanoyl-L-homoserinelactone induced the formation of colonies in *M. aeruginosa*. The production of microcystin toxin was greater with 3-oxododecanoyl-homoserine lactone and hexanoyl–homoserine lactone.

All these results conclusively show that *QS* is a common phenomenon in bacteria and that autoinducers molecules accomplish specific functions in the pathogenic processes. Therefore, the understanding of the chemical factors involved could give valuable tools for the discovery of new drugs and pharmacologic treatments. Recently, the role of several drugs, including acetaminophen, induce *QS* in *Klebsiella pneumoniae*. Thus, it is probable that substances structural or functionally related to these lactones and from human activity (industry, agrochemical, drug, and food) can trigger the blooms and bacterial resistance to antibiotics.

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40. 2D-QSAR Studies of Dopamine Transporter Inhibitors (DAT) Using OPS and GA Variable Selection Approaches

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Today, drug abuse has developed into a social problem and begun to demand specific measures from different social sectors and government agencies all over the world. Despite significant efforts made toward relevant mechanistic targets, such as the dopamine transporter (DAT), the development of pharmacotherapeutic treatments of psychostimulant abuse has remained a challenge so far. Using a set of 49 2-[(diphenylmethyl)sulfanyl]ethanamines described as DAT inhibitors, 2D-QSAR/PLS studies were performed using two different approaches of variable selection: Ordered predictors selection (OPS) and genetic algorithm (GA). All structures were optimized at the B3LYP/6-311G++(d,p) level of theory. The molecular descriptors were obtained in the Dragon 6 program (topological, geometric, molecular, and constitutional) and GaussView 05 (electronic). Both models were formed by two latent variables. Model 1 (OPS) was constructed with four molecular descriptors (GATS3m, Mor15p, SpMin3_Bh(s), and HOMO-1), while six (Mor13m, CATS2D_09_LL, RDF110u, RDF085m, Mor24s, and RDF010s) were required to obtain model 2 (GA). The models can be considered reasonably different: In model 1,electronic features predominate, whereas in model 2, steric and geometric effects do. The overall test indicated that models 1 and 2 have equivalent predictive ability (Average r^2_m Overall = 0.730 versus 0.710 and Delta r^2_m Overall = 0.122 versus 0.151). However, model 1 is simpler (it has only four descriptors, which facilitates its interpretation), presents more relevant information used in the construction of its two latent variables (75.99% versus 64.07%), and its calibration is more significant than that of model 2 ($F_{n,n-p-1}$ = 115.814 versus 80.888, for the same tabled *F* value, where *n* = 36, and *n*-*p*-1 = 3.256, with alfa = 0.05). Considering these results, although model 2 may also be considered a good result, model 1, obtained using the OPS approach for variable selection, may be considered more reliable for prediction purposes. This result is in agreement with good results previously obtained using the OPS methodology.

41. 3D and 4D-QSAR of Dopamine Transporter Inhibitors (DAT) Using the LQTA-QSAR Approach

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At present, drug abuse has developed into a social problem and begun to demand specific measures from different social sectors and government agencies all over the world. Despite significant efforts, the development of pharmacotherapeutic treatments of psychostimulant abuse has remained a challenge so far. Using 49 2-[(diphenylmethyl)sulfanyl]ethanamines described as dopamine transporter (DAT) inhibitors, 3D and 4D-QSAR studies were performed using the LQTA-QSAR approach. This method, initially created for the construction of models based on conformational sampling profiles obtained by molecular dynamics, has been adapted to allow studies based on only a single optimized geometry. In both studies, Coulomb and Lennard-Jones descriptors were used, which were generated with the NH_3^+ probe atom. The variable selection was carried out using the ordered predictors selection (OPS) method in the free QSAR modeling program. Both regression models were constructed using PLS. The models were formed by two latent variables, which in turn were constructed based on five Coulomb and six Lennard-Jones descriptors in both cases. These results appear to be related to the presence of hydrophobic and polar amino acid residues at the binding site. The overall test indicated that the 3D model (Average r^2_m overall = 0.849, Delta r^2_m overall = 0.082) is slightly superior to the 4D model (Average r^2_m overall = 0.762, Delta r^2_m overall = 0.129). To test the models for purposes of prediction, a virtual screening based on 2D similarity (Dice 60%) was performed in the ZINC15 Database, and 12 compounds were selected. The Euclidean applicability domain test showed that all compounds presented a normalized mean distance inferior to 1. On the other hand, the PRI test indicated that predictions by both models can be considered moderate, an adequate result considering that all compounds selected in the VS are structurally related to the data set but do not present a test for the inhibition of the DAT described in literature.

42. Supporting Compound Optimisation in Not-For-Profit and Academic Research

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The not-for-profit and academic sectors have become important sources of novel drug candidates, particularly for neglected and developing world diseases or niche indications. Drug discovery projects in these sectors are often conducted on a collaborative basis, pooling resources and experience across multiple research groups and using contract research organisations as appropriate. Several software platforms have been developed to facilitate the secure sharing of data across organisations, but here we will discuss software approaches that focus on using these data to guide decisions regarding the selection and design of high-quality compounds.

Given the limitations of the resources available to projects in these sectors, it is important to quickly focus on chemical series and leads with the best possible chance of success downstream. Enabling this requires a combination of capabilities, including visualisation and analysis of project data, interpretation of structure–activity relationships, and predictive modelling to guide the design of new compounds for synthesis and testing. In this talk, we will describe the underlying methods and illustrate how they can be linked to platforms for sharing of data, to facilitate collaborative approaches to drug optimisation.

We will illustrate the approaches described here with an application to a drug discovery project targeting malaria. We will demonstrate how an integrated application of computational approaches can help to guide the multi-parameter optimisation of efficacy against drug sensitive and resistant strains of plasmodium parasites, as well as absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties.

43. 1,2,3-Triazole-oxazolidinone Derivatives as Inhibitors of the Plasminogen System *

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**** Selected Poster for the Best Poster Award***

The Plasminogen system is known for being responsible for clot dissolution through fibrin degradation. The inactive zymogen plasminogen is activated by t-PA or u-PA to form the active plasmin, which exerts the proteolytic activity to degrade fibrin, as well as other ECM proteins.
High throughput screening methods have led to the discovery of a new family of antifibrinolytic drugs, based on derivatives of substituted 1,2,3-triazole-oxazolidinones. Of the 13 different molecules successfully synthesized, two showed high inhibition activity in coagulation in vitro tests.
Specific in vitro assays have been used to study t-PA and Plasmin activities separately. Results suggest a simultaneous inhibition of both t-PA and plasmin. The inhibition mechanisms for each target have been proposed after studying the binding interactions through docking computational analysis.
These findings open the door to further explore this family of compounds as therapeutic candidates to prevent extreme blood loss during certain types of surgery, severe menstruations, and other bleeding related conditions.

44. A Novel Class of Cholinesterases- and NMDA Receptor-Directed Multi-Target Anti-Alzheimer Agents

Andreea Larisa Turcu [1], F. Javier Pérez-Areales [1], Marta Barniol-xicota [1], Caterina Pont [1], Deborah Pivetta [1], Alba Espargaró [2], Manuela Bartolini [3], Angela De Simone [4], Vincenza Andrisano [4], Belén Pérez [5], David Vílchez [6], F. Javier Luque [6], Raimon Sabate [2], Francesc X. Sureda [7], Santiago Vazquez [1], Diego Muñoz-Torrero [1]

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The multi-target-directed ligands (MTDLs) approach is a promising modern strategy specifically developed for the treatment of disorders with a complex pathological mechanism. One of such disorders is Alzheimer's disease (AD), a multifactorial neurodegenerative disease, where the absence of an effective therapy and its multifactorial nature call for hybrids that combine distinct pharmacophoric moieties to confer multiple activities. Our vast experience in the development of multi-target anti-Alzheimer compounds containing acetylcholinesterase (AChE) inhibitor pharmacophores [1–6] and the discovery of novel benzohomoadamantane derivatives as potent NMDA receptor antagonists [7] led us to design a novel class of multi-target hybrids. Thus, we will present the rational design, synthesis, and multi-target biological profiling of a new hybrid that combines a benzoadamantane scaffold with the potent cholinesterases inhibitor 6-chlorotacrine, with both moieties being connected by oligomethylenic linkers of two different lengths and different attachment positions. The modulation of both human AChE and human butyrylcholinesterase (BChE), the glutamate NMDA receptor antagonistic activity, and the beta-amyloid peptide and tau anti-aggregating activity were studied. The novel compounds do behave as multi-target drugs in vitro with high potency, especially against AChE and BChE (up to 44- and 24-fold, respectively, more potent than 6-chlorotacrine), and NMDA receptors (up to 2-fold more potent than memantine), thereby emerging as a promising new class of multi-target anti-Azlheimer agents.

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45. A Study on Neonatal Intake of Oleanolic Acid and Metformin in Rats (Rattus norveticus) with Metabolic Dysfunction: Implications on Lipid Metabolism and Glucose Transport

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Metabolic syndrome can be traced back to early developmental stages of life. The aim of this study was to investigate the sub-acute and long-term effects of neonatal oral administration of oleanolic acid and metformin on FFAs and genes associated with lipid metabolism and glucose transport using a neonatal rat model. In the 1st study, 7-day-old pups were grouped into control—distilled water (DW); oleanolic acid (60 mg/kg), metformin (500 mg/kg), high fructose diet (20% w/v, HF), oleanolic acid (OA) + high fructose diet (OA + HF), and Metformin + high fructose diet (MET + HF) groups. The pups were treated for 7 days and then terminated on postnatal day (PD) 14. In the 2nd study, pups were treated similarly to study 1 and weaned onto normal rat chow and plain drinking water on PD 21 until they reached adulthood (PD112). Tissue and blood samples were collected for analysis. Measurement of the levels of free fatty acids was done using GC-MS. QPCR was used to analyze the expression of glut-4, glut-5, fas, acc-1, nrf-1, and cpt-1 in the skeletal muscle. The results showed that HF accelerated accumulation of saturated FFAs within skeletal muscles. The HF-fed neonatal rats had increased stearic acid, which was associated with decreased glucose, suppressed expression of glut-4, glut-5, nrf-1, and cpt-1 genes, and increased expression of acc-1 ($p < 0.01$) and fas. The OA + HF and MET + HF groups had increased mono- and polyunsaturated FFAs, oleic, and octadecadienoic acids than the HF group. These unsaturated FFAs were associated with increased glut-4, glut-5, and nrf-1 ($p < 0.01$) and decreased acc-1 and fas ($p < 0.05$) in both the OA + HF and MET + HF groups. The present study shows that neonatal oral administration of oleanolic acid and metformin potentially protects against the development of fructose-induced metabolic dysfunction in the rats in both short- and long-time periods.

46. Amaryllidaceae Alkaloids from *Zephyrantes carinata* and Their Evaluation as Cholinesterases (AChE and BChE) Inhibitors

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The subfamily Amaryllidoideae within of the Amaryllidaceae family has an exclusive group of compounds called Amaryllidaceae alkaloids. Galanthamine, the most known Amaryllidaceae alkaloid, is approved by the FDA as an inhibitor of the enzyme acetylcholinesterase (AChE) for the palliative treatment of Alzheimer Disease (AD). However, butyrylcholinesterase (BChE) contributes critically to cholinergic dysfunction associated with AD. Thus, the development of novel therapeutics may involve the inhibition of both cholinesterase enzymes. *Zephyranthes carinata*, a species of the Amaryllidaceae family, has been reported to have inhibitory activity against cholinesterases. In order to determine the enzymatic inhibition potential, the major alkaloids of bulbs and leaves of *Z. carinata* were evaluated in both AChE and BChE. A purification and characterization process was made using different chromatographic and spectrometric techniques, and the inhibitory activity was evaluated with the Ellman method. Alkaloidal extracts of bulbs and leaves exhibited an inhibitory activity with IC_{50} values of 5.8 ± 0.2 and 8.7 ± 0.3 μg/mL, respectively, against AChE. Further, bulb extract showed IC_{50} values of 77.9 ± 3.4 μg/mL against BChE. Amaryllidaceae alkaloids hamayne, pseudolycorine, galanthine, criasbetaine, tazettine, lycoramine, hippeastidine, galanthamine, trisphaeridine, 3-epimacronine, haemanthamine, lycorine, and vittatine were purified and evaluated for their AChE and BChE inhibitory activities. Lycoramine (galanthamine type) presented the lowest IC_{50} value in AChE (17 ± 0.7 μg/mL), and trisphaeridine (narciclasine type) showed the lowest IC_{50} value in BChE (33.1 ± 3.6 μg/mL). Combined major alkaloids (>10%) were analyzed to observe synergistic behavior. The mixture alkaloids lycoramine and galanthine presented IC_{50} values of 14.55 ± 1.0 μg/mL against AChE, and the lycoramine, trisphaeridine, and vittatine mix presented IC_{50} values of 38.42 ± 3.4 μg/mL in BChE. These results showed prominent inhibitory activity against AChE and BChE enzymes, indicating their potential as agents for treating AD through a combined strategy.

47. Biological Evaluation of a Mitochondrial Phosphoenolpyruvate Carboxykinase Inhibitor

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Phosphoenolpyruvate carboxykinase (PEPCK) is a key enzyme in gluconeogenesis, catalyzing the decarboxylation of oxaloacetate to phosphoenolpyruvate. In eukaryotes, there are two isozymes present either in the cytosol (PEPCK-C, PCK1) or in the mitochondria (PEPCK-M, PCK2). PCK2 is highly expressed in pancreatic β-cells, where it contributes to the regulation of the TCA cycle flux by coupling it to mitochondria GDP recycling. This flux has been shown to regulate glucose stimulated insulin secretion (GSIS).

In order to obtain high purity and efficacy compounds, we synthetized, through lineal synthesis, a group of C-8 modified 3-alkyl-1,8-dibenzylxanthines, starting from 6-aminouracil. These compounds were described as potent PEPCK-C inhibitors by Roche. Structural and docking analysis of both PEPCK isoforms showed that both enzymes are structurally very close and might be inhibited by the same family of inhibitors.

The lead inhibitor (INH-2) was compared with 3-mecaptopicolinic acid, a classic PCK1 inhibitor, in a kinetic assay, and the results confirmed the cross-inhibitory capacity between PCK1/PCK2 and their increased potency as inhibitors. Moreover, we studied PCK2 target engagement of our candidates through cellular thermal shift assays (CETSA).

INH-2 successfully inhibits GSIS in INS-1 cell line through PEPCK-M inhibition, validating the cross-inhibition between PEPCK-C and PEPCK-M. Furthermore, this compound inhibited insulin secretion In vivo, on an impaired glucose tolerance test (IGTT) in mice.

48. Automated Microwave-Assisted Synthesis for PET Radiochemistry

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Microwave heating has proven to be a highly effective source for a wide range of chemical transformations, increasing reaction rates and yields [1,2]. Due to the shorter half-life of commonly used radionuclides for positron emission tomography (PET), such as ^{18}F and ^{68}Ga, reduction of reaction times and increase of yields is of critical importance.

The main goal of this work was to implement an automated method using microwave technology to synthesize radiopharmaceuticals for PET imaging.

No-carrier-added [^{18}F]-fluoride was produced by an IBA Cyclone® 18/9 cyclotron. $^{68}GaCl_3$ was obtained from a GalliaPharm® Ge-68/Ga-68 Generator from Eckert & Ziegler Radiopharma. Radiolabeling reactions were performed in a PET-Wave Discover® from CEM and an automatic module Trasis AllinOne®.

Microwave heating was adapted to an existing automated setup in our site and tested for the production of commonly used radiopharmaceuticals, such as [^{18}F]FDG, [^{18}F]FDOPA, [^{68}Ga]DOTANOC, and [^{68}Ga]PSMA.

Time for azeotropic drying of [^{18}F]KF and subsequent [^{18}F] fluorination of mannose triflate were performed with a radiochemical yield (RCY) of 78% and a reduction in time of 40%. Fluorination of 6-nitroveratraldehyde was performed with an RCY of 100% with a time reduction of 60%. We also tested the setup in the radiolabelling of peptides (DOTANOC and PSMA) with ^{68}Ga, achieving 100% reaction in 1.5 minutes, instead of the 10 minutes with conventional heating (reduction of 85%).

We optimized the automated radiolabeling of commonly used PET radiopharmaceuticals using microwave technology. Radiosynthesis was performed with good RCY in a substantially shorter reaction time than conventional heating. These results suggest that the incorporation of microwave technology could provide a substantial advantage in the synthesis of radiopharmaceuticals with application in PET.

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49. Computational Study of Benzimidazole Derivatives as Potential Antifungal Drugs

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The infection of fungal diseases has increased in these last years due to the rise in immunodeficient patients, the resistance to current drugs, and the lack of design of new and efficient molecules against those pathogens. On the other hand, benzimidazole derivatives have shown inhibitory activity to Cryptococcus Neoformans fungal with MIC 50 (minimal inhibitory concentration) of 31.2, 15.6, and 7.8 µg/mL for DV, NRE, and ACH type compounds, respectively. In order to find new compounds with benzimidazolic scaffolds by modifying functional chemical groups that can have better antifungal activity, we carried out a computational study using molecular docking, molecular dynamics, and MMGBSA (mechanics generalized born and surface area continuum solvation) to screen the affinity of benzimidazolic ligands with two *Mycobacterium Turberculosis* enzymes (pdb 1E9X y 3IW2). Those enzymes are involved in the synthesis of ergosterol, which is an important component in the cell membrane wall. The accomplishment of the study presented is a good agreement between the computational model and the experimental results and delivered promising compounds as candidate inhibitors based on benzimidazolic scaffold. Furthermore, other studies have reported that compounds with inhibitory capacity to enzyme 1E9X are also potent inhibitors of mycobacterial growth. For this reason, the new benzimidazole derivatives found in this work can be used as effective drugs against infections caused by fungal and Mycobacterium Turberculosis at the same time. Our proposal is based on the fact that ligands have a tight bind with the pocket of enzyme 3IW2.

50. D-Ring Opened Oxime Analogues of 17-Beta Estradiol as Estrogen Receptor Subtype-Selective Ligands

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In an effort to improve the subtype-selectivity and agonist potency, we have designed and developed a series of D-ring opened oxime analogues of 17-beta estradiol as estrogen receptor ligands useful in treating ER and/or estrogen-associated diseases. Based on the scaffold hopping methodology, we planned to deconstruct the D-ring of E_2 to make it jump into a new chemical space of E_2-related ER ligands. We also envisioned that conformational flexibility induced by ring opening might enhance the ligand's ability to recognize the slight difference in the binding cavities between the two receptors. In addition, we introduced oxime functionality as a bioisoster of the 17-OH group. In view of stereoelectronic reasons, oxime could be a good candidate for the 17-hydroxyl group. The biological activities and the selectivity for the receptor subtype of new oxime analogues as well as their prototype D-ring opened E_2 analogues have been studied by radioligand competitive binding assay and cell-based transcription assays. Among the derivatives synthesized, oxime analogue seemed the most potent and efficacious ER_b-agonist with an EC_{50} of 82.9 pM and an E_{max} of 133% at 100 nM. Its EC_{50} for ER_a-mediated transcription was 2.9 pM, which is about 8-fold more potent than E_2. D-ring opened *O*-benzyl oxime analog showed remarkably high ER_a-binding affinity with 1.8 nM of *K*i value and ER_a subtype-selectivity up to 30. A similar result was obtained on the transcription activation study. In summary, our approach is successful in generating the novel and promising scaffold with a subnanomolar concentrations range of EC_{50} and greater efficacy than E_2, and we propose that they may serve as the lead structure to develop subtype-selective ER ligands useful in treating ER and estrogen-associated diseases.

51. Design and Synthesis of Immunostimulating Mannosylated Muropeptide Analogs Containing 2-Aminoadamantane-2-carboxylic Acid *

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**** Selected Poster for the Best Poster Award***

Muramyl dipeptide (MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine) is known as the smallest synthetic adjuvant molecule capable of replacing whole *Mycobacteria* in Freund's adjuvant [1]. Numerous MDP derivatives were synthesized with the aim to avoid MDP unwanted side-effects. Many of them have therapeutic potential, including clinical use [2]. A very important parameter in the improvement of pharmacological properties of MDP is lipophilicity, e.g., it eliminates drawbacks caused by poor macrophage penetration and rapid elimination. On the other side, mannose receptors (MR), present on immunocompetent cells (such as macrophages and dendritic cells), are considered to be pattern-recognition receptors and responsible for the binding, among others, of mannosylated antigens or relevant biologically active molecules containing mannose, thus affecting the immune reactions [3]. Up to now, our research was directed towards desmuramyl peptides which contain adamantylglycine and mannosylated adamantylglycine moieties bound to the essential part of MDP, L-Ala-D-*iso*Gln [4]. Here, we present the design and synthesis of novel mannosylated muropeptide analogs containing 2-aminoadamantane-2-carboxylic acid. Prepared desmuramyl peptides have lipophilic 2-aminoadamantane-2-carboxylic acid attached at the N-terminus of desmuramy dipeptide core and mannose connected to the tripeptide over a glycolyl linker. Immunostimulating activities of prepared compounds will be evaluated in the mice model using ovalbumin as an antigen and compared with previously prepared derivatives.

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52. Design of Novel GPR6 Inverse Agonists Using a Fragment Replacement Scaffold Hopping Approach

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The orphan G protein coupled receptor 6 (GPR6) is a cannabinoid-related Class A GPCR. It is highly expressed in the central nervous system and exhibits high constitutive activation of adenylyl cyclase. Several research groups have demonstrated that GPR6 represents a possible target for the treatment of neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's diseases. Several patents claim the use of a wide range of pyrazine derivatives as GPR6 inverse agonists for the treatment of Parkinson's disease and other dyskinesia syndromes.

Using cyclic AMP accumulation assays in *h*GPR6-CHO cells as a readout, the most potent GPR6 pyridopyrazine inverse agonist compounds identified thus far have been found to display IC_{50} values in the low nanomolar range. A subset of these compounds was used here as starting points for the design of novel potent GPR6 inverse agonists using a core hopping approach.

In parallel with the core hopping studies, we employed a recently constructed homology model of the GPR6 inactive state. The X-ray crystal structure of the Sphingosine-1-phosphate receptor 1 (S1P1) receptor structure was used as the template and the conformational memories technique was used to explore the conformational consequences of sequence differences between S1P1 and GPR6. The most potent GPR6 pyridopyrazine inverse agonists were docked in the resultant GPR6 inactive state model, first as tests of the model. Once we had identified the binding site of each inverse agonist, we used this site to identify amino acid sites in proximity that we can use to build additional interactions for ligands output from core hopping studies above. ADME properties and the absence of PAINS will also be considered for the hit selection process. These potential GPR6 chemotypes may serve as research tools for further understanding the biological role of this orphan receptor.

53. Design, Synthesis, and Pharmacological Evaluation of Novel Quinolone Aryl Sulfonamide Derivatives as Potent GPR55 Antagonists *

Paula Morales [1,2], Laura Figuerola-Asencio [1], Dow H Hurst [2], Pingwei Zhao [3], Mary E Abood [3], Patricia H Reggio [2], Nadine Jagerovic [1]

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**** Selected Poster for the Best Poster Award***

The orphan Class A G-protein-coupled receptor GPR55 has been proposed as a potential member of the cannabinoid receptor family. This receptor has been implicated in numerous physiopathological conditions, such as metabolic disorders, inflammatory and neuropathic pain, regulation of vascular functions, bone physiology, cancer, and motor coordination.

Diverse studies point towards the phospholipid LPI (lysophosphatidylinositol) as the endogenous ligand for GPR55. In addition, diverse chemical entities, endogenous, phytogenic, and synthetic cannabinoid ligands among them have been shown to modulate this receptor. Nonetheless, pharmacological inconsistencies and the lack of potent and selective GPR55 ligands are delaying the exploitation of such a promising therapeutic target.

In an effort to identify GPR55 ligands, a high-throughput, high-content screening discovered the quinolone aryl sulfonamide ML193 (CID1261822) as an antagonist of this receptor [1]. Using our GPR55 inactive state model [2,3], docking studies of this compound and its analogs helped us to rationalize key structural features involved in ligand–receptor binding. On this molecular basis, we have designed novel quinolone sulfonamide derivatives with optimized potency and efficacy. These novel molecules compounds are being synthesized and evaluated using a β-arrestin recruitment assay in CHO cells overexpressing human GPR55 and barr2-GFP.

In summary, we pursued a combination of structure–activity relationship development and molecular modeling studies to identify novel potent GPR55 antagonists that may serve as new tools for studying GPR55. [Support: NIH RO1 DA045698]

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54. Epigenetic and PPI Targeted Libraries from Life Chemicals

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Herein, we report our novel epigenetic and PPI screening libraries in response to a growing interest in these topics for discovery of novel drugs against emerging molecular targets.

Epigenetic inhibitors have been found to be very effective in cancer treatment. Epigenetic regulation of gene expression is not caused by changes in the nucleotide DNA sequences and implies modulation of chromatin structure (epigenetic marks). As epigenetic changes are dynamic, they can be reversed by epigenetic inhibitors. We designed three new Epigenetic Libraries using both structure- and ligand-based approaches. Virtual screening against various confirmed targets resulted in preparation of the Epigenetic Targeted Library and SIRT Targeted Library. Meanwhile, our Epigenetic Focused Library comprises compounds selected with 2D Fingerprint Similarity search.

Protein–protein interactions (PPIs) are involved in many important biological processes in life, so their regulation can be crucial for the treatment of numerous diseases. Low molecular weight PPI inhibitors able to selectively and potently modulate protein–protein interactions have recently reached clinical trials. Inspired by this promising breakthrough, we have prepared several PPI Focused Libraries of potential PPI inhibitors using various features within the ligand-based approach. A machine learning method (Decision Tree), known to be a useful tool to identify a PPI inhibitor profile, was used to predict which compounds could affect PPI. We also designed PPI Focused Libraries based on 2D Similarity Search (to Timbal DB or Binding DB, Pubmed DB). Alternatively, docking search against PDZ domain-containing proteins provided PPI Inhibitors Libraries.

55. Exploring the Chemical Universe

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Small molecules have been the major source of new drugs. However, the target space is limited, and overused in-stock collections are less and less capable of providing new chemical entities (NCEs). In a joint venture, Enamine and BioSolveIT built the world's largest chemical space and made it ultra-fast searchable. The new product, called REALSpaceNavigator, comprising 3.7 billion compounds, allows for efficient hit exploration, from finding previously unknown analogues to scaffold hopping. The technology supports fast similarity searching (about 3 min only on a 4 GB RAM laptop) in the space and convenient analysis of the. The chemical space, encoded with more than 100 highly validated synthesis protocols and in-stock building blocks, provides an escape from the availability bias of current stock screening collections towards IP-free areas. Compounds selected from this space will be synthesized in 3 weeks with an exceptional success rate of 80% and above. However, the 3.7 billion compounds are just a first step towards exploring the universe of molecules. How far can we go?

56. FOXM1 Inhibitors as Potential Theranostic Agents: Initial Steps in the Validation of FOXM1 as a Positron Emission Tomography (PET) Probe for Triple-Negative Breast Cancer Detection

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The FOXM1 transcription factor controls the expression of essential genes related to cell cycle progression and replication; under normal physiological conditions, its expression is significantly decreased in terminally differentiated cells, but it is abnormally activated in most (if not all) malignant cells. During the last three years, our research group has worked on the development of novel (still experimental) FOXM1 inhibitors. We hypothesize that binding interactions exerted by FOXM1 inhibitors could not only inhibit its transcriptional activity but also serve as PET-based imaging probes, specifically, ^{18}F-based imaging. We prepared derivatives from **FDI-6** (reference FOXM1 inhibitor) and evaluated them as FOXM1 inhibitors using the electrophoretic mobility shift assay (EMSA) method. To determine their ability to exert in vitro FOXM1 expression inhibition and anti-proliferative activity, we used a triple-negative breast cancer cell line (MDA-MB-231). We chose compound **FDI-AF**, which was able to dissociate the FOXM1-DNA complex (EMSA results), also inhibited FOXM1 expression levels, and showed anti-proliferative activity in MDA-MB-231 cells. We designed a suitable method to radiolabel it with a fluorine-18 atom and prepared **^{18}F-FDI-AF** in 60% radiochemical yield. The cell uptake results in MDA-MB-231 cells for **^{18}F-AF -FDI** showed that it reached an overall 500% of radioactivity/mg of cell protein at 2 h, and based on the internalization experiments, we found that around 55% of the radioactivity/mg of protein was internalized in cells and 45% was membrane bound. The inhibiting uptake with the reference compounds **FDI-AF** and **FDI-6** in MDA-MB-231 cells, showed that IC_{50} required to block the 50% of **^{18}F-AF–FDI**'s cell uptake were 50 and 31 (micromolar) respectively, which suggests specificity towards FOXM1. We have established the initial steps to validate FOXM1 as a potential probe for PET imaging and submit these preliminary results, suggesting that it might be possible to use transcription factors to develop "theranostic agents".

57. Hydrophobic Waters in Bromodomains

Serena G. Piticchio [1,2], Miriam Martínez-Cartró [1,2], Salvatore Scaffidi [1,2], Sergio Rodríguez-Arévalo [3], Andrea Bagán [3], Ainoa Sánchez-Arfelis [3], Carmen Escolano [3], Carles Galdeano [1,2], Xavier Barril [1,2]

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Targeting epigenetic proteins is a rapidly growing area for medicinal chemistry and drug discovery. Recent years have seen an explosion of interest in developing small molecules binding to bromodomains due to their implication in cancer, inflammation, and a plethora of diseases. Several small-molecule inhibitors and degraders that target bromodomains have entered the clinic, and many more are increasingly being used as chemical probes to describe bromodomain biology. From a structural point of view, crystallographic studies of bromodomains describe, as a common feature, five water molecules as an integral part of the acetyl–lysine binding pocket. These water molecules are essential in druggability and are described as a functional part of the protein [1,2].
In this framework, we focused our attention on the description of the hydrophilic/hydrophobic character of these molecules, which seem to create a favorable environment for the recognition of hydrophobic groups. To this end, and following fragment-based drug design techniques, here we describe a new family of small molecules with a 5-phenylthiazolo[2,3-*c*][1,2,4]triazol nucleus and probe the water site with various substituents at the 3-position endowing hydrophilic or hydrophobic properties. In this work, we present the theoretical calculations, the synthesis of the new compounds, the results of differential scanning fluorimetry (DSF) and isothermal titration calorimetry (ITC), and the crystal structures of three of our compounds with the target protein. The study sheds light on the counterintuitive behavior of the water molecules in this particular environment.

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58. Identification of Inhibitors of the Anti-Infective Target DXS Using Dynamic Combinatorial Chemistry *

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**** Selected Poster for the Best Poster Award***

Antibiotic resistance is one of the biggest threats to humankind [1,2]. This global problem is aggravated by bacteria developing new resistance mechanisms and the emergence of extremely drug-resistant strains of the pathogens. In this alarming situation, novel targets for which inhibitors with an unprecedented mode of action can be developed are urgently required.

Our study aims at the development of selective and potent inhibitors of the important and underexplored anti-infective target DXS. This enzyme from the 2C-methyl-D-erythritol 4-phosphate pathway is entirely absent in humans but is essential for medically relevant pathogens (e.g., *Plasmodium falciparum, Mycobacterium tuberculosis, Pseudomonas aeruginosa,* and *methicillin-resistant Staphylococcus aureus*). Despite substantial efforts dedicated to the discovery of inhibitors for DXS, to date, very few active compounds have been reported, and none of them fulfill the requirements as an ideal candidate for further development. To address these issues and maximize the chances of success, we are using a combination of structure-based drug design and target-directed dynamic combinatorial chemistry (tdDCC) as hit-identification strategies for the first time for DXS. To expand structural diversity and obtain potent and selective inhibitors of DXS, we designed the dynamic combinatorial library for acyl hydrazone formation. Different heterocyclic hydrazides and aldehydes were chosen based on calculated estimated affinity using SeeSAR for all possible acyl hydrazone products. Biochemical evaluation of five hit compounds amplified in a first round of tdDCC experiment against *M. tuberculosis* DXS and *D. radioduran* DXS afforded inhibitors with IC_{50} in the range of 49–190 mM [3]. Further improvement of the activity by a more tailor-made DCC-library and by SAR of hits obtained is underway.

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59. *In Vitro* Assessment of the Neuroprotective and Antioxidant Properties of New Benzimidazole Derivatives as Potential Drug Candidates for the Treatment of Parkinson's Disease

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Oxidative stress is related to the pathogenesis of many neurodegenerative disorders, including Parkinson's and Alzheimer's disease. The inability of the neuronal cells to maintain redox balance leads to free radicals accumulation, mitochondrial dysfunction, and neuronal injury.

The neurons are highly sensitive to oxidative stress due to stronger dependence on oxidative phosphorylation, exposure to high concentrations of oxygen, and accumulation of metal ions during aging which increase the generation of reactive oxygen species. Other factors are the presence of easily oxidized polyunsaturated fatty acids and the relatively poor concentrations of antioxidants.

A series of new benzimidazole hydrazones containing hydroxy and methoxy substituents were synthesized as analogues of Melatonin—a known antioxidant with neuroprotective action. The neurotoxicological potential of the compounds was assessed, and the derivatives demonstrating the most prominent effects were studied for neuroprotective properties in different in vitro models: H_2O_2-induced oxidative stress in neuroblastoma SH-SY5Y cells and 6-hydroxydopamine (6-OHDA) induced neurotoxicity in rat brain synaptosomes. As markers of oxidative damage, SH-SY5Y cell viability, synaptosomal viability, and intra-synaptosomal content of GSH were used.

For further investigation of antioxidant properties, in vitro spectrophotometric model systems have been used. The antiradical activity against the stable free radicals ABTS and DPPH has been estimated, as well as the capability of the derivatives to decrease the level of molecular damage of biologically important molecules upon ferrous iron-induced oxidative molecular damage. The obtained data revealed that the tested compounds demonstrate a protective effect and capability to decrease the concentration of stable free radicals. Their potency depends of the used radical, oxidisable substrate, the type, and the position of the structural modification in the evaluated molecular structure.

Different possible mechanisms, such as hydrogen atom transfer (HAT), single-electron transfer (SET-PT), and sequential proton loss electron transfer (SPLET), were studied by DFT methods.

60. Induction of Biofilm Formation in *Klebsiella pneumoniae* by Acetaminophen. *Quorum Quenching* Compounds to Overcome the Resistance

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Bacterial resistance to antibiotics is a serious world health problem; consequently, there is a high recurrence of nosocomial infections. For this reason, the WHO has launched a warning to look for new molecules and mechanisms of action against bacterial pathogens. On the other hand, *Quorum Sensing* is a bacterial communication process involved in pathogenesis and virulence; it is mediated by several types of molecules, mainly lactones, quinolones, peptides, and boron derivatives, among others. In addition, the use of inhibitors or *Quorum Quenching* compounds as an alternative to overcome this type of resistance has been proposed. Thus, we consider that some medicines might also be responsible for bacteria resistance acting like inducers of *Quorum Sensing*. Therefore, in this work, we aimed to determine the role of some drugs in the resistance of *Klebsiella pneumoni*ae and to establish the effects of several natural products against this pathogen bacteria.

Effectively, some drugs, especially acetaminophen, induced the biofilm formation, the synthesis of an autoinducer lactone, the colonization of urethral catheters, and increased the resistance of biofilm to gentamicin. However, several compounds were also able to counteract the effect of acetaminophen and even weaken the formed biofilm.

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61. Multicomponent Reaction-Based Trimethoprim Analogues as Potential Antibiotics for Resistant Bacteria

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Aminoimidazoles are relevant scaffolds in medicinal chemistry. They can be assembled by condensation of aldehydes, isocyanides, and α-amino azines through the Groebke–Blackburn–Bienaymé multicomponent reaction (GBBR). Our group recently discovered a novel approach for "selective multiple GBBRs", yielding *N*-fused polyheterocyclic scaffolds. The process could be exploited to generate screening libraries for medical applications.

Trimethoprim (TMP, antibiotic), methotrexate (anticancer), and lamotrigine (anticonvulsant) are commercial drugs with a diaminopyrimidine motif. This core can be involved in selective multiple GBBRs, and novel scaffolds can be decorated with up to four diversity points to access more efficient analogues. TMP is commonly prescribed for the treatment of lower urinary tract infections and acute diarrhoea/bacterial dysentery in humans and animals. However, despite its efficiency, TMP-resistance is frequently reported. Therefore, facilitated access to structurally tunable analogues is utterly important. In this regard, a set of TMP analogues was synthesized through multiple GBBR procedures, using a variety of aldehydes and isocyanides, and their bioactivity was determined. SAR studies and impact on TMP-resistance will be discussed.

62. Natural Products as Sources of New Drugs for the Regulation of Mast Cell Activation

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Taking into account that the identification of novel molecules for the effective treatment of inflammatory and immune diseases is one of the main present medical needs and one of the major goals of the pharmaceutical industry, the aim of our work is intended to provide new therapeutic strategies and a deeper understanding of the mechanism of action of new drugs related to such disorders. Our research team has shown that some natural and synthetic lactones developed by our laboratory, as well as phenols isolated from virgin olive oil, inhibit mast cell activation induced by immune and non-immune pathways, thus acting as mast cell stabilizers. Recently, we have started to explore whether the application of these mast cell stabilizers will be useful for prevention and/or treatment of mast cell-mediated disorders.

Diseases investigated include: Peptic ulcer, neuropathic pain, tumor development, multiple sclerosis, and allergic asthma. Our laboratory investigates the role of mast cells in such pathologies and the pharmacological regulation of mast cell activation by conducting studies on animal and human mast cells, and by analyzing specimens derived from patients with mast cell disorders. Biochemical, chemical, cell biology, molecular biology, and a variety of microscopic techniques were used, as well as animal models for the investigated diseases in which mast cells are involved. These studies may lead to an increased understanding of these disorders and may contribute to new preventive measures, diagnosis, and treatments.

63. New Diarylureas as Activators of the Heme-Regulated EIF2α Kinase for the Treatment of Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) has reached epidemic proportions. Unfortunately, current therapies for T2DM are suboptimal, and thus, there is an urgent need to develop more effective treatments. Fibroblast growth factor 21 (FGF21) has recently emerged as a therapeutic strategy for treating T2DM due to its antidiabetic effects, and this has led to the development of FGF21 long-acting analogs. However, these compounds require subcutaneous injection and have shown some serious side effects, mainly due to their prolonged pharmacodynamic effect compared with native FGF21. Therefore, there is a need for orally available approaches to enhance FGF21 native production.

We have lately demonstrated that activation of the heme-regulated eIF2α kinase (HRI) by intraperitoneal administration of some already known *N*,*N*'-diarylureas increases FGF21 levels in liver, leading to an improvement of glucose intolerance and hepatic steatosis in mice fed a high-fat diet.

Encouraged by our findings, we aimed to design and synthesize new *N*,*N*'-diarylureas suitable for oral administration. Thus, a large series of compounds have been developed and evaluated in a human hepatocyte cell line in culture. Further in vitro profiling (mice and rat microsomal stability, solubility, cytotoxicity) and pharmacokinetics have allowed us to select a candidate for in vivo efficacy studies. Proof-of-concept studies have demonstrated that oral administration of our lead compound improves glucose intolerance, hepatic steatosis, and hypertriglyceridemia in mice fed a high-fat diet.

Overall, the results presented herein suggest that orally bioavailable HRI activators induce FGF21 production and may be of clinical interest for the treatment of T2DM and other metabolic disorders.

64. Novel Sortase A Inhibitors to Counteract Gram-Positive Bacterial Biofilms

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Sortase A (SrtA) is a membrane enzyme responsible for the covalent anchoring of surface proteins on the cell wall of Gram-positive bacteria. Nowadays, it is considered an interesting target for the development of new anti-infective drugs which aim to interfere with important Gram-positive virulence mechanisms. Along the years, we studied the anti-staphylococcal and anti-biofilm activity of some natural and synthetic polyhalogenated pyrrolic compounds, called pyrrolomycins. Some of them were active on Gram-positive pathogens at a μg/mL range of concentration (1.5–0.045 μg/mL) and showed a biofilm inhibition in the range of 50–80% [1–3].

In light of these encouraging results, herein we present our efforts in the design and synthesis of novel pyrrolomycins. To dispose of a sufficient amount for the in-depth in vitro investigation, we developed an efficient and easy-to-use microwave synthetic methodology. All compounds showed a good inhibitory activity toward SrtA, in accordance with the molecular modelling studies, having IC_{50} values ranging from 130 to 300 μM comparable to berberine hydrochloride, our reference compound. Particularly, the pentabromo-derivative exhibited the highest capability to interfere with biofilm formation of *S. aureus* with an IC_{50} of 3.4 nM. This compound was also effective in altering *S. aureus* murein hydrolase activity, responsible for degradation, turnover, and maturation of bacterial peptidoglycan, and involved in the initial stages of *S. aureus* biofilm formation [4].

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65. Pursuit of Novel Hedgehog Pathway Inhibitors by Targeting BRD4 *

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**** Selected Poster for the Best Poster Award***

The hedgehog (Hh) signaling pathway is a major regulator governing cell proliferation and differentiation. Its aberrant activation after birth drives initiation and maintenance of numerous types of tumors, such as basal cellular carcinoma (BCC) and medulloblastoma (MB). The first generation of Hh inhibitors has been approved for use in the clinic but with limited success and suffering from drug resistance. Epigenetically targeting the transcriptional factor Gli, downstream of the Hh pathway, through the bromo and extra C-terminal (BET) bromodomain 4 (BRD4), has recently emerged as a more effective approach both to treat Hh-driven cancers and to overcome drug resistance of the already-approved Hh pathway inhibitors. Recently, we conducted a medicinal chemistry approach by starting from a clinical BRD4 inhibitor and finely tailoring it as new Hh pathway inhibitors. Herein, we report the results of our new inhibitors on the relevance between BRD4 and Gli, and their in vitro and in vivo antitumor efficacy in the treatment of medulloblastoma.

66. Searching for Selective Scaffolds against *Plasmodium falciparum* Glucose-6-Phosphate Dehydrogenase 6-Phosphogluconolactonase

Antonio Viayna [1], David Vílchez [1], Javier Vázquez [1,2], Susana Pérez-Benavente [3], José Manuel Bautista [3,4], Francisco Javier Luque [1]

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Malaria is a parasitic disease caused by *Plasmodium* spp., being one of the major causes of death worldwide with two-hundred million new infections and hundreds of thousands of deaths in 2015. Despite the important advances in its prevention and treatment, its resistance to current drug therapies is still a serious risk in its eradication.

There is urgency in finding novel targets and drugs operating by novel mechanisms, avoiding cross-resistance to classical antimalarials. In this context, the bifunctional enzyme Glucose-6-phosphate dehydrogenase 6-phosphogluconolactonase appears to be a promising therapeutic target due to its crucial role in regulating the PPP pathway (pentose phosphate pathway), which is the major source of redox potential in *Plasmodium falciparum*.

In the last few years, our group detected a specific mutation between the human and the *Plasmodium falciparum* form in the binding site of Glucose-6-phosphate (G6P), the endogenous ligand of Glucose-6-phosphate dehydrogenase (G6PD). This mutation involves the substitution of an Arginine (human) by an Aspartate (parasite), which allowed us to create a validated in-house homology model of *Pf*G6PD.

Based on this result, the group has focused their efforts, through different molecular modelling techniques, in the discovery of selective scaffolds against *Pf*G6PD. Current efforts address the development of a complete structural model of the bifunctional enzyme, which may offer novel opportunities to develop molecules capable of inhibiting this relevant enzyme.

67. Toward an Innovative Treatment of Alzheimer's Disease: Design of MTDLs Targeting Acetylcholinesterase and α-7 Nicotinic Receptors *

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**** Selected Poster for the Best Poster Award***

Alzheimer's disease (AD) is a complex and progressive neurodegenerative disorder. The available therapy is limited to symptomatic treatment, and its efficacy remains unsatisfactory. In view of the prevalence and expected increase in the incidence of AD, the development of an effective therapy is crucial for public health. Since the therapeutic paradigm "one compound–one-target" has shown its limits in the treatment of AD, new strategies are emerging to overcome the lack of efficiency of the current pharmacotherapy in the past decade. The most promising is the multitarget-directed ligands (MTDLs) strategy. This project consists of the development of new multifunctional agents, which will act simultaneously on the different players in AD pathology by combining an AChE inhibitory activity based on the structures of a well-known AChE inhibitor (RIvastigmine) with an α-7 nAChR activation. Indeed, α-7 nAChRs were also put forward as potent targets for the treatment of central nervous system (CNS) diseases, such as AD. Because of their distribution and abundance in the CNS, the α-7 subtypes are potential therapeutic targets for this disorder.

68. Understanding the Mechanism of Direct Activation of AMP-Kinase: Towards a Fine Allosteric Tuning of the Kinase Activity

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This research deals with the regulation of the AMPK activity by direct activators, such as compound A-769662. AMPK is a key enzyme to maintain the cellular energy homeostasis, as it regulates the levels of ATP, being an important target to metabolic diseases like obesity or diabetes MT2. It is formed by 3 subunits α, β, and γ. The activation mechanism of A-769662 is of particular interest, because it activates AMPK independently of α-Thr172 phosphorylation, the β-Ser108 being phosphorylated. Under these circumstances, binding of A-769662 enhances the AMPK activity up to >90-fold (PDB 4CFF) [1–3].
We have recently studied the chain of events implicated in the binding of this ligand to the activating binding site, which is located between the α and β subunits of AMPK. MD simulations of AMPK were run for apo, holo, and holo+ ATP systems. For each system, we ran three independent MD simulations up to 1 μs. The impact of the activator binding was studied by different analysis, such as essential dynamics and evaluation of conformational entropies, among others [4].
We concluded that A-769662 acts as a molecular glue, making an effective connection between β- and α-subunits that pre-organizes the ATP-binding site, favouring the binding of ATP, and explaining the increase of the AMPK activity. These findings pave the way to explore the structural features that underline the different sensitivity of AMPK isoforms to A-769662, i.e., try to discern why A-769662 is only active in the α2β1γ1 isoform, while other compounds are active with isoform β2.

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69. Discovery of Small Molecule Inhibitors of APOBEC Enzymes by High-Throughput Screening

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The APOBEC family of enzymes catalyze the water-mediated deamination of cytosines to uracils in single-stranded DNA. This innate immune defense mechanism against pathogenic DNA is comprised of 7 structurally similar APOBEC3 enzymes bearing notable differences in their deaminase domain architectures, oligonucleotide substrate preferences, and subcellular localizations. Unfortunately, viruses and cancer cells can exploit the pro-mutagenic capacities of APOBEC enzymes to promote genome mutations that contribute to disease progression and the evolution of drug resistance mutations. Therefore, we hypothesize that small molecule chemical probes of APOBEC enzymes will yield valuable reagents for mechanistic biological studies of these important proteins, as well as serve as lead compounds for the development of future therapeutics. Using an *in vitro*, microplate-based fluorescent ssDNA deamination assay,[1] our collaborative research team has identified early chemical inhibitors of APOBEC enzymes using high-throughput screening of small molecule libraries.[1-3] This poster will focus on the discovery of first-in-class, small molecule APOBEC inhibitors through our efforts, as well as present some unanticipated discoveries from our hit triage studies.

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70. Inhibition of LPS-Induced PGE_2 Production by Arylsulfonamide Derivatives via the Selective Inhibition of mPGES-1 Enzyme

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Microsomal prostaglandin E synthase-1 (mPGES-1) is responsible for the massive prostaglandin E_2(PGE_2) formation during inflammation. Increasing evidence reveals mPGES-1 inhibitors as a safe alternative to nonsteroidal anti-inflammatory drugs. Recently, we reported that a novel series of phenylsulfonyl hydrazide derivatives could reduce LPS-induced PGE_2 levels in RAW 264.7 macrophage cells via an inhibition of the mPGES-1 enzyme. However, a few of the phenylsulfonyl hydrazide derivatives showed poor metabolic stability in liver microsomes. In order to identify new mPGES-1 inhibitors with improved metabolic stability, therefore, a series of arylsulfonamide derivatives has been synthesized and biologically evaluated against PGE_2 production and the mPGES-1 enzyme. Among them, MPO-0186 inhibits the production of PGE_2 (IC_{50} = 0.20 μM) in A549 cells via inhibition of mPGES-1 (IC_{50} = 0.49 μM in a cell-free assay) together with high selectivity over both COX-1 and COX-2. A molecular docking study theoretically suggests that MPO-0186 could inhibit PGE_2 production by blocking the PGH_2 binding site of the mPGES-1 enzyme. Furthermore, MPO-0186 demonstrated good metabolic stability in human liver microsomes and no significant inhibition observed in clinically relevant CYP isoforms.

71. Promising Approach to Inhibit *E-coli* FimH Adhesion by C-Linked Mannosides *

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**** Selected Poster for the Best Poster Award***

Antagonists of the uropathogenic *Escherichia coli* type-1 fimbrial adhesin (FimH) are recognized as attractive alternatives for antibiotic therapies and prophylactic strategies against acute and recurrent bacterial infections.

In this study, *C*-linked α-D-mannopyranosides possessing aromatic aglycons were investigated to fit within the hydrophobic pocket of the FimH Tyrosine gate (Tyr48-Tyr137). The results were summarized into a set of structure–activity relationships to be used toward FimH-targeted inhibitor design. Alkene linkers afforded improved affinity and inhibitory potential, because they could provide favorable binding interactions with hydrophobic side chains located in the middle of the tyrosine gate.

Of particular interest was a *C*-mannoside derivative, prepared by a Heck reaction between a family of aryl iodides and *C*-allyl α-D-mannopyranoside. One of them, an *ortho*-substituted biphenyl aglycone, showed an affinity enhancement in the nM range. Docking of its high-resolution NMR solution structure to the FimH adhesin indicated that it could present its *ortho*-substituted phenyl ring directly in contact with isoleucine-13 (Ile13), located in the clamp loop that undergoes conformational changes under shear force exerted on the bacteria upon binding to its receptor. Molecular dynamic simulations confirmed that a subpopulation of the C-mannoside conformers was able to interact in this secondary binding site of FimH, thus unraveling a new mode of binding, useful in the design of potent inhibitors against the *E. coli* adhesion.

72. The Ameliorating Effect of Phenylsulfonamide Derivatives on Scopolamine-Induced Memory Impairment in Mice via Inhibition of mPGES-1

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Our previous research showed that a novel series of phenylsulfonyl hydrazide derivatives reduced LPS-induced PGE_2 levels in RAW 264.7 macrophage cells via an inhibition of mPGES-1 enzyme. As a continuous work, new phenylsulfonamide derivatives (**5a**-**5k**) as methylene analogues of phenylsulfonyl hydrazide derivatives, including **MPO-0063**, were synthesized and biologically evaluated in vitro. Among synthetic compounds, **5a** (**MPO-0112**) showed decreased inhibitory activity against PGE_2 production (IC_{50}: 0.34 μM) compared to **MPO-0063** (IC_{50}: 0.04 μM) but inhibited the mPGES-1 enzyme (IC_{50}: 7.37 μM) similar to **MPO-0063** (IC_{50}: 0.10 μM) together with excellent selectivity over COX-enzymes (COX-1 and 2). According to recent studies on the close correlation between up-regulation of mPGES-1 and Alzheimer's disease, we investigated whether **5a** could ameliorate scopolamine-induced memory impairment using the passive avoidance test. The memory impairment-ameliorating effect of **5a** (1.0 mg/kg, p.o.) was found to be effective, comparable to that of donepezil (5 mg/kg, p.o.) as a positive control. On the other hand, **5a** exhibited little or weak AChE and BuChE inhibitory activity, which implies that **5a** could ameliorate scopolamine-induced memory impairment by inhibiting mPGES-1 enzyme instead of cholinesterase enzymes. In addition, **MPO-0112** exhibited a favorable in vitro CYP profile, which is suggestive of no potential drug–drug interactions. Therefore, these overall results suggest that **5a** as a selective mPGES-1 inhibitor may be a novel therapeutic agent for diseases associated with cognitive deficits, such as Alzheimer's disease.

73. Chiral Resolution of a New Agent against Memory Impairment Connected to Neurodegenerative Diseases

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Nowadays, the incidence of neurodegenerative diseases is increasing, and these disorders will become one of the main challenges for medicine and public health in future years. Particularly, memory loss characterizes many neurodegenerative pathologies, and it is often related to low levels of cyclic adenosine monophosphate (cAMP). During recent years, researchers have highlighted that inhibiting phosphodiesterase type 4 (PDE4)-mediated hydrolysis of cAMP could represent a powerful tool to contrast memory impairment. In 2017, we identified GEBR-32a as a new molecular entity able to full inhibit PDE4D, a specific PDE4 isoform. GEBR-32a was able to restore memory in an Alzheimer's disease rodent model without causing emesis, which is the typical undesired effect of the major part of PDE4 inhibitors. Hence, it has been selected for preclinical studies. Since stereoselectivity is known to play a role for receptor ligands, to investigate the influence of chirality on the interaction of compounds with PDE4, the chiral resolution of GEBR-32a was performed. On the basis of our previous experience, the racemate GEBR-32a was resolved by chiral high-performance liquid chromatography (HPLC), this approach being an effective way for both the analytical and the preparative separations of chiral compounds. The analytical screening was performed on several chiral chromatographic columns, under different elution conditions, adopting isocratic modes. The optimized method was transferred into a semipreparative scale, and enantiomers were isolated within good enantiomeric excess. Configuration assignment studies are ongoing.

74. Mechanisms of Inhibitory Effects of Polysubstituted Pyrimidines on Prostaglandin E_2 Production

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The pyrimidine heterocycle represents an elemental structural motif of numerous drugs. We have synthesized a large series of original derivatives possessing different substituents at C-2, C-4, C-5, and C-6 positions of the pyrimidine ring. The vast majority of prepared pyrimidines inhibit prostaglandin E_2 (PGE_2) production as revealed in vitro in the lipopolysaccharide (LPS)-stimulated mouse macrophages [1,2]. A number of them are effective at sub-micromolar concentration. The compounds are devoid of cytotoxic effects. They do not inhibit activities of phospholipase A2 (sPLA2), cyclooxygenases COX-1 and COX-2, and important enzymes in the PGE_2 biosynthesis pathway. A plausible explanation for the mechanism of PGE_2-inhibitory effects of pyrimidines is provided by findings showing substantial inhibition of activity of the terminal enzyme in PGE_2 formation, i.e., microsomal prostaglandin E_2 synthase-1 (mPGES-1). The IC_{50}s characterizing the potential of compounds to reduce mPGES-1 activity on one site and LPS-induced PGE_2 production on the other one are statistically significantly correlated.
Pyrimidine derivatives exhibit anti-inflammatory activity in vivo, as demonstrated by the significant reduction of carrageenan-induced paw oedema in rats. The findings suggest that pyrimidine inhibitors of mPGES-1 activity and consequent PGE_2 production may be considered as promising candidates for further preclinical research and development of novel non-steroidal anti-inflammatory drugs.

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75. Amyloid Pan-Inhibitors: Can a Single Compound Treat All Conformational Diseases?

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Amyloids are ubiquitous protein aggregates that are present in all organisms, playing physiological or pathological roles. In humans, amyloid aggregation can be responsible for several conformational diseases, including both non-neurological (such as type 2 diabetes) and neurodegenerative diseases (like Alzheimer's and Parkinson's disease). In the last few years, a lot of effort has gone into finding effective therapies against conformational diseases. Among these, therapies focused on inhibiting amyloid aggregation have become of greater importance. Despite the fact that amyloids display common structural characteristics, amyloidogenic proteins have traditionally been investigated separately, in order to find putative selective inhibitors for specific conformational diseases.

Herein, we suggest the idea that a single compound, inhibitor of a specific amyloid-prone protein, can also be efficient in inhibiting other amyloidogenic proteins, thus becoming a generic anti-aggregating agent with a promising potential in the treatment of conformational diseases. Three anti-amyloid inhibitors, previously developed by our group, were tested against thirteen distinct amyloid-prone proteins that encompass all the major types of amyloid-based disorders. Using a simple but contrasted cell-based screening procedure [1,2], we demonstrated that these compounds can act as potential aggregation pan-inhibitors, thus opening a great perspective in the development of a universal treatment for conformational diseases [3].

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76. Antibacterial *N*-Arylcinnamamides as Anti-inflammatory Agents

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A series of ring-substituted *N*-arylcinnamamides was prepared, characterized, and investigated for their antimicrobial efficacy in detail. Several of these derivatives showed strong activities, especially against Gram-positive bacteria and mycobacteria; they were able to increase the antibacterial activity of clinically used antibiotics with different mechanisms of actions. They were even able to inhibit the growth of staphylococcal biofilm and disrupted mature biofilm in concentrations close to MICs. It was found that the activity of the arylcinnamamides is bactericidal. No cytotoxic effect on human cells was observed for the most effective compounds [1,2]. Thus, the investigated cinnamamides may be considered as promising compounds for future research.
As derivatives of cinnamic acid are known for their anti-inflammatory and antioxidant effects [3,4], the cytotoxicity of the most effective compounds was tested on chondrocytes, and no inhibition of proliferation was found up to 100 μg/mL concentration. Thus, based on the concepts of polypharmacology, multifactorial diseases, and multitarget drugs and the abovementioned results, the compounds were investigated on their ability to inhibit cartilage damage caused by inflammation processes. This contribution is focused especially on the anti-inflammatory investigation of ring-substituted *N*-arylcinnamamides in relation to the induction of apoptosis and expression of pro- and anti-inflammatory markers. Structure–activity relationships and the supposed mechanism of action are discussed.

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77. Can Short Peptides Be Inhibitors of Serum Amyloid A Protein Aggregation?

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One of the approaches in the design of anti-amyloid drugs is to find compounds that are able to hamper self-association of amyloidogenic protein molecules. There is an idea that aggregation inhibitors can be sought in the amyloidogenic sequence itself and prevent protein oligomerization due to shielding the interaction site [1,2]. We have focused our studies on inhibitors of the aggregation of serum amyloid A protein (SAA). SAA is an acute phase protein whose increased production occurs in response to injury, inflammation or infection [3]. Prolonged high concentration of circulating SAA may lead to its aggregation and accumulation of deposits of the protein in the cellular matrix of various organs and finally cause a death of the affected tissues and organs [4]. In our research, we used the 1–5 fragment of SAA as a lead compound to design aggregation inhibitors. We modified the sequence of this fragment by introducing various natural and unnatural aromatic residues. The inhibitory capacity of the saa1-5 analogs was studied using two fragments of the most amyloidogenic *N*-terminal region of the SAA protein, SAA1-12 and SAA1-27. In order to determine the impact of the inhibitor on the model peptide structure and the type and morphology of aggregates, circular dichroism spectroscopy, quantitative chromatographic analysis of the soluble fraction, and transmission electron microscopy were employed, respectively.

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78. Influence of Synthesis Conditions on the Applicative Physiochemical Properties of Drug–Polyester Conjugates for Controlled Drug Delivery

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Coupling of active pharmaceutical substances (API) with macromolecular species is one of the most investigated approaches applied to control the fate of API after administration. The drug, covalently or physically attached to polymeric structures with different architecture, usually expresses altered pharmacokinetics and can be delivered to the specific site of action. Biodegradable polyesters like poly-ε-caprolactone (PCL) or polylactic acid (PLA) are suitable materials for preparation of a drug delivery system and can be covalently attached to drug molecules during polymer synthesis via ring-opening polymerization. In the present study, selected APIs were employed as initiators of PCL and PCL-PLA random copolymer synthesis. Covalent bonding between polymer chain and drug was confirmed via electrospray ionization time-of-flight mass spectrometry. Polymerization kinetics in varied temperatures and catalyst concentrations were investigated. Selected polymerization products were processed using the solvent evaporation method to obtain submicron solid spherical structures. Influence of the reaction temperature and the concentration of catalyst, 2-ethyl hexanoate, on the reaction kinetics was confirmed. Decomposition of micromatrices in physiological-like conditions was monitored via dynamic light scattering measurements of their hydrodynamic diameter, whereas changes of pH linked to hydrolytic carrier degradation were monitored in parallel. Microparticles containing atactic lactic acid monomers exhibited a significantly higher decomposition rate in comparison to conjugates with PCL only.

79. New Compounds against Leishmania infantum from Eremurus persicus

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Leishmaniasis is a complex of vector-borne diseases caused by several species of the protozoan parasite of the genus *Leishmania*. This disease affects 12 million people in the world, and its burden is particularly felt in undeveloped and developing countries. To date, a vaccine has not been discovered yet; thus, chemotherapy remains the only effective way to treat every form of the disease. However, many available drugs have the drawback of causing both serious side effects and toxicity, and resistance. Accordingly, there is an urgent need of new effective drugs.
Within a wider research project aimed at discovering new bioactive compounds from nature, for several years we have focused our attention on plants from the Asian region and particularly on *Eremurus persicus.* Following a bio-guided fractionation approach, we isolated (*R*)-Aloesaponol III 8-methyl ether (*R*)-ASME, characterized by a remarkable antiprotozoal effect against *L. infantum* (IC50 = 73 µg/mL). However, (*R*)-ASME has a very low solubility both in water and in aqueous buffers, and therefore, the biological assays can be difficult to perform and time-consuming, since a high number of replicates are needed. Starting from (*R*)-ASME as a *hit compound*, we designed new water-soluble (*R*)-ASME-derivatives. Taking into account its structural features and molecular reactivity, molecular modification strategies have been planned, including conjugation of (*R*)-ASME with either amino acid (AA), or with PEG moiety. The latter approach allowed us to obtain a stable and water soluble salt. The assays against *Leishmania infantum* are still in progress.

80. New TRPV1 Antagonists as Candidates for Effective Anticonvulsant and Antinociceptive Agents *

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**** Selected Poster for the Best Poster Award***

Epilepsy is recognized as one of the most common neurological disorders with a high risk of drug resistance. Notably, about one-third of the patients with epilepsy are not responsive to pharmacological treatment. Thus, the search for new, more effective anticonvulsants with a novel mechanism of action is undoubtedly necessary. The most recent neurobiological studies implicate central TRPV1 receptors in the induction of epileptic seizures. Moreover, it is suggested that TRPV1 desensitization is one of the crucial mechanisms of action responsible for the anticonvulsant activity of cannabidiol (CBD), which was proven to be effective against drug-resistant epilepsy. Bearing in mind the aforementioned facts, we developed in our recent studies a series of chemically original TRPV1 antagonists. Their structures were designed as integrated hybrids that join on the common chemical template the structural fragments of anticonvulsants identified by our team in the previous studies and known TRPV1 antagonists (described in the literature). As a result, these compounds revealed potent anticonvulsant activity in the preclinical studies using the most widely employed animal seizure models, namely, the maximal electroshock (MES) test, and the psychomotor 6 Hz (32 mA and 44 mA) seizure model in mice. In addition, selected substances demonstrated potent effectiveness by decreasing pain responses in formalin-induced tonic pain, in capsaicin-induced neurogenic pain, as well as in oxaliplatin-induced neuropathic pain in mice.

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81. Novel Dual Ligands Targeting Sigma1 Receptor and Acetylcholinesterase Endowed with Antioxidant Properties

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Neurodegenerative disorders (e.g., Alzheimer's diseases, Parkinson's disease, multiple sclerosis) represent one of the main therapeutic challenges of our time. Considering their complex nature, the multi-target paradigm is gaining great consensus in the search for small molecules that are able to counteract these pathologies. Among the numerous molecular pathways, impairments of the cholinergic system have been strictly correlated to several neurodegenerative diseases, and acetylcholinesterase (AChE) inhibitors have shown to be effective against such disorders by restoring the physiological amount of acetylcholine. On the other hand, Sigma1 Receptor (S1R) agonists are known to exert neuroprotective activity and thus, they are gaining attention as promising pharmacological tools in the fight against neurodegenerative disorders. Moreover, cell damage caused by reactive oxygen species (ROS) is generally considered a hallmark of neurodegeneration conditions. Given these premises, we tested this scenario with the preparation of a small compound library aimed at the identification of new small molecules endowed with both S1R affinity, anti-AChE activity, and antioxidant properties. The designed compounds are based on a common arylalkylaminoketone scaffold, which bears the structural elements of our developed S1R agonist **RC-33**, the well-known AChE inhibitor Donepezil and the antioxidant molecule Curcumin. The obtained compounds were tested for a preliminary biological evaluation: The affinity and selectivity towards S1R was determined, as well as their inhibition of AChE and the antioxidant profile. Two *hit* compounds emerged from this work, and they will undergo further structure optimization in order to achieve viable tools for the treatment of neurodegenerative pathologies.

82. Physicochemical and Pharmacokinetic Properties of New Dual-Acting Compounds for the Treatment of Mental Disorders

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Physicochemical and pharmacokinetic properties of compounds marked with acronyms PQA-AZ-4 and PQA-AZ-6 were studied using experimental methods. Selected compounds (dual-active effective inhibitors of phosphodiesterase (PDE) 10A and serotonin 5-HT_{1A} and 5-HT_7 receptor ligands) in prescreening pharmacological studies revealed antipsychotic-like, antidepressant-like, and anxiolytic activities. The liquid chromatographic–tandem mass spectrometric method with electrospray ionization (LC/ESI-MS/MS) system was calibrated and validated for lipophilicity studies. Lipophilicity was assessed from the y axis intercept (y_0) of the linear regression line of $\ln((t - t_0)/ t_0)$ against % concentration of organic eluent. Finally, lipophilicity was calculated using a linear regression equation of the logP value against y_0 values obtained for the reference compounds in the same experimental conditions. Next, the thermodynamic aqueous solubility of selected compounds was detected with UPLC detection. The LC/ESI-MS/MS system was used for the simultaneous determination of PQA-AZ-4 and PQA-AZ-6 in mouse plasma, hippocampus, striatum, and frontal cortex, developed and validated according to GLP procedures. Finally, drug-likeness properties of selected compounds were evaluated using a predictive bioavailability radar model from the SwissADME web tool. The descriptors of physicochemical properties (lipophilicity, size, polarity, solubility, flexibility, and saturation) for selected compounds were projected next on the optimal range for each property to be considered drug-like.

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83. Synthesis, *In Vitro* Profiling, and *In Vivo* Efficacy Studies of a New Family of Multitarget Anti-Alzheimer Compounds

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Simultaneous modulation of several targets or pathological events with a key pathogenic role is a promising strategy to tackle thus far difficult-to-cure or incurable multifactorial diseases, such as Alzheimer's disease (AD). In this scenario, multitarget compounds, i.e. single molecules that hit several targets, are superior to other multitarget strategies that are based on the use of more than one drug (drug cocktails, fixed-dose combinations), in terms of simpler drug development and better patient compliance, efficiency, and safety.

In the frame of our research line devoted to the development of novel anti-AD drug candidates, we have recently prepared a new class of multitarget compounds, which were designed by combining pharmacophoric moieties of a known antioxidant agent (7-methoxy-2,2-dimethylchroman-6-ol (CR-6)) and an acetylcholinesterase (AChE) inhibitor (6-chlorotacrine), to primarily address two important pathological events of AD, namely oxidative stress and cholinergic deficit. Here, we present the synthesis of three short series of CR-6–chlorotacrine hybrids, featuring different linker functionalities (amide, inverse amide, or amine) and lengths, and their in vitro biological activities against AChE, butyrylcholinesterase, BACE-1, and β-amyloid and tau protein aggregation, their antioxidant activity, and BBB permeability. We will also show the results of the in vivo efficacy studies of two selected compounds in double transgenic APP/PS1 mice, a well-established mouse model of AD (behavioral studies, effects on amyloid pathology and oxidative stress).

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84. Synthesis and Biological Evaluation of Aminobisphosphonates—Analogues of Incadronate

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Bisphosphonates had been found to act as strong metal ion complexing agents with useful industrial and household applications. Today, they are a prominent class of organophosphorus drugs used to slow or prevent bone damage and, thus, are standardly applied for the prevention and treatment of osteopenia and osteoporosis. Despite this important medicinal use, they display a variety of physiologic activities, which make them promising anticancer, antiprotozoal, antibacterial, and antiviral agents. Consequently, their biological properties are still intensively studied, and these studies deliver promising drug candidates.

The three-component reaction between amines, triethyl orthoformate, and diethyl phosphite is perhaps the simplest and most commonly used reaction providing structurally variable aminomethylenebisphosphonates. It typically yields a complex mixture of products, which are not separated but immediately hydrolyzed with HCl to lead to the desired bisphosphonic acids in moderate to good yields.

A series of *N*-alkyl- and *N*-cycloalkylaminomethylenebisphosphonates, analogues of incadronate, have been obtained and evaluated for their anti-proliferative activity against a model mouse macrophage J774E. These cells originated from identical precursors as osteoclasts [1]. The in vivo studies were carried out on sheep. These animals were chosen because of sheep's similarity with humans in weight, bone and joint structure, and mechanisms of bone regeneration [2]. The metabolic rate of sheep (based on oxygen consumption per gram of body weight) is closer to that of man than that of a rat or dog model. Two of the most active compounds were used for fracture healing in sheep with steroid-induced osteoporosis. They demonstrated a mild antiosteoporotic activity that was documented using bone histopathology.

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85. The Content of Phenolic Compounds and Flavonoids in Medical Herbs Depending on the Area of Growth

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The aim of this study was to investigate the process of accumulation of antioxidants in the herbal extracts depending on the stage of the herbal vegetation and the natural conditions of the growth.

A spectrophotometric analysis based on the complex creation of flavonoids with aluminum ion was performed. Previously, the calibration curves were designed for rutine (to measure the content of flavonoids) and for gallic acid (to measure the overall content of phenolic compounds). Moreover, the following formula was used to calculate the content of flavonoids and phenolic compounds. We investigated herbs growing in the Goris region of Armenia (1370 m above sea level) and herbs growing in the Central Botanic Garden of Minsk, Belarus (220 m above sea level). The optic density of the solutions was measured by SPECTRO UV-11. Flavonoids were detected by 410 nm waves and phenols were detected by 760 nm waves.

From the 5 investigated herbs (*Fragaria vesca* L., *Artemisia vulgaris* L., *Trifolum paratense* L., *Cichorium intybus* L., and *Taraxacum officinale*), extractions of the first four ones contained more phenols and flavonoids while growing in the Goris region of Armenia compared to those from Belarus. The exception was Taraxacum officinale, the content of phenolic compounds of which was higher in the Belarus species. This can be explained by the fact that herbs growing in the highland are subject to more extreme environmental factors, such as higher levels of ultraviolet radiation and insolation, lower temperatures, etc. To resist the aforementioned abiotic conditions, herbs develop a survival strategy which expresses itself via higher synthesis of phenolic compounds.

The majority of extractions of investigated medical herbs growing in the highland contain higher levels of phenolic compounds and flavonoids compared to those growing in lower geographic locations.

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