Program and Abstracts

24th Scientific Congress of the Austrian Pharmaceutical Society (ÖPhG)

12-14 September 2015

Pharmaziezentrum UZA2, Althanstrasse 14, 1090 Vienna, Austria

Conference Chairmen

Thierry Langer

Helmut Viernstein

Organizing Commitee

Julia Kirchebner

Ernst Urban

Daniela Krizan

Sabrina Werner

Scientific Commitee

Rudolf Bauer, Graz

Franz Bucar, Graz

Verena Dirsch, Wien

Gerhard Ecker, Wien

Rosa Lemmens-Gruber, Wien

Manfred Ogris, Wien

Judith Rollinger, Wien

Hermann Stuppner, Innsbruck

Time Schedule

	Saturday, Sep 12, 2015	Sunday, Sep 13, 2015	Monday, Sep 14, 2015
8:00			Plenary Lecture Pharmaceutical Chemistry
8:20			
8:40			
9:00		Plenary Lecture Pharm Technology	Oral Presentation PC-1
9:20			Oral Presentation PC-2
9:40			Oral Presentation PC-3
10:00		Oral Presentation PT-1	Coffee Break
10:20		Oral Presentation PT-2	Oral Presentation PC-4
10:40			Oral Presentation PC-5
11:00		Coffee Break	Oral Presentation COMP-3
11:20		Oral Presentation PT-3	Plenary Lecture Pharmacology
11:40		Oral Presentation COMP-1	
12:00		Oral Presentation COMP-2	
12:20		Oral Presentation PK-1	Closing Ceremony
12:40		Lunch Break	
13:00			
13:20		Plenary Lecture Clinical Pharmacy	
13:40			
14:00			
14:20		Oral Presentation PK-2	
14:40		Oral Presentation PKN-1	
15:00	Registration	Oral Presentation PKN-2	
15:20		Coffee Break	
15:40		Poster Session	
16:00			
16:20			
16:40			
17:00	Opening Ceremony	Plenary Lecture Pharmacognosy	
17:20	Keynote Opening Lecture		
17:40			
18:00			
18:20	Welcome Get-Together		
18:40			
19:00		Congress Dinner Restaurant Wolf	
19:20			
19:40			
20:00			
20:20			
20:40			
21:00			

Scientific Program

Plenary Lectures

Keynote Opening Lecture

Prof. Dr. Peter Hammann, Sanofi (Frankfurt)
Blast from the past: Will resurrection of under-exploited antibiotics solve the crisis?

Pharmaceutical Technology

Prof. Dr. Achim Göpferich, University of Regensburg
Ocular Drug Delivery: Indispensable or Only Nice to Have?

Clinical Pharmacy

Mag. pharm. Dr. Silvia Hetz, Klinikum Wels-Grieskirchen
Aktuelle Herausforderungen in der Krankenhauspharmazie

Pharmacognosy

Prof. Dr. Sergey B. Zotchev, University of Vienna
Harnessing Bacteria for Drug Discovery: From Bioprospecting to Synthetic Biology

Pharmaceutical Chemistry

Dr. Peter Nussbaumer, Lead Discovery Center GmbH (Dortmund)
Challenges in Early Drug Discovery and Medicinal Chemistry: Where to Go?

Pharmacology

Prof. Dr. Walter Berger, Medical University of Vienna
Rational Cancer Therapy: Fact or Fiction?

Oral Communications

PT-1: Dr. Monika Müller, University of Vienna
Prebiotic effect of fructans depending on polymerization degree and structure

PT-2: Priv.-Doz. Dr. Hubert Rein, University of Bonn
Melt extrusion as a continuous production process for solid dosage forms

PT-3: Dr. Sharareh Salar-Behzadi, RCPE GmbH Graz
Taste Masked Formulations with Advanced Performance

COMP-1: Dr. Daria Goldmann, University of Vienna
In Silico Perspective of TRPV1 Modulation

COMP-2: Dr. Wu Shengqian, RCPE Graz
Influence of in vitro pulmonary permeability on in silico prediction of oral inhalation products

COMP-3: Mag. Eva-Maria Plessl-Zangerl, University of Vienna
Towards a structural understanding of Cantú disease associated gating perturbations in the K_{ATP} potassium channel

PKN-1: Dr. Ulrike Grienke, University of Vienna
hERG channel inhibiting alkaloids from Nelumbo nucifera: How safe is the "Lotus Leaf Diet"?

PKN-2: Mag. pharm. Christina E. Mair, University of Vienna
In vivo characterisation of voacangine - a hERG blocking iboga alkaloid from Voacanga africana

PK-1: Dr. Erich Leitner, Austrian Chemical Society
Personalized Medicine – Compounding of Preparations

PK-2: Dr. Chi Huu Nguyen, University of Vienna
NF-κB dependent MMP1 expression in MDAMB-231 breast cancer spheroids causes paracrine activation of PAR1 and lymph endothelial barrier disintegration in vitro

PC-1: Prof. Dr. Wolfgang Robien, University of Vienna
A few Remarks on the Quality of Published Carbon-NMR Data

PC-2: Dr. Heinrich Orsini-Rosenberg, Bruker Austria
Modern Practice for Material Identification and Quality Control in Pharmacies and Pharmaceutical Industry

PC-3: Dr. Daniela Digles, University of Vienna
An Open PHACTS Knime workflow to collect compound data from public databases

PC-4: Dr. Daniel Merk, Goethe-University Frankfurt
Development and preclinical characterization of partial farnesoid X receptor agonists for metabolic disorders

PC-5: Dr. Jennifer A. Weiß, University of Vienna
Synthesis and enantioseparation of eight new amphetamine derivatives by indirect gas chromatography-mass spectrometry after derivatization with (R)-(+)-α–methoxy-α-trifluoromethylphenylacetic acid (MTPA) or with (R)-(-)-menthylchloroformate or by HPLC-UV

Poster Presentations

	Presenter	Title
P-1	M. Grandits	A molecular dynamics approach to study P-glycoprotein in complex with propafenone-type ligands
P-2	E. Hellsberg	Elucidation of the structure of a strigolactone ABC-transporter in Petunia hybrida
P-3	S. Jain	Structure based classification for BSEP/ABCB11 Inhibitors using comparative structural modeling of human BSEP
P-4	A. Knox	Design and synthesis of new diethylstilbestrol derivatives as anti-breast cancer agents
P-5	E. Kotsampasakou	Predicting hyperbilirubinemia by combining extended connectivity fingerprints (ECFPs) and liver transporter inhibition profiles
P-6	R. Lesyk	5-Ylidene-4-thiazolidinones as new anticancer agents: pro et contra
P-7	D. Kaminskyy	Design of the new antitrypanosomal agents bearing 4-thiazolidinone core
P-8	C. Lorenzer	Generation and evaluation of DARPin-siRNA conjugates for receptor-specific tumor targeting
P-9	V. Obermoser	Antitumour active cobalt alkyne complexes derived from acetylic salicylic acid: studies on impact of fluorination and chlorination of Co-ASS
P-10	I. Perkovic	Synthesis and characterization of novel primaquine ureas and semicarbazides
P-11	T. Seidel	Conformational Sampling with iCon: Algorithm and Performance Assessment
P-12	A. Taschauer	Linear polyethylenimine based nanocarriers for gene therapy: single nanoparticle tracking analysis, production upscale and microspraying
P-13	M. Taschner	Quantification of the relative percentage distribution of THCA and Δ9-THC in herbal cannabis seized by Austrian police: Stability tests at different storage temperatures
P-14	T. Weigl-Pollak	Propafenone variations: Probing selectivity between P-glycoprotein and BCRP
P-15	A. Weninger	Novel Zeise-type complexes with aspirin substructure as potential COX-inhibitors
P-16	M. Wieder	Testing the robustness of pharmacophore models using molecular dynamic simulations
P-17	E. Wurzer-Priester	Synthesis of amino acid variations for the generation of short α-melanocyte stimulating hormone analogues
P-18	V. Bochkov	Selective inhibition of proinflammatory action of oxidative stress via non-antioxidant mechanisms
P-19	A. Cseke	Using flow cytometry for evaluation of structure-activity relationships of ABCB1 and ABCG2 inhibitors
P-20	M. Delijewski	A comparative effect of nicotine and UV radiation on melanization process in HEMn-DP and HEMn-LP melanocytes
P-21	S. Gehrig	3D Imageable spontaneously metastasizing orthotopic cancer models and a new paradigm in anti cancer drug development

	Presenter	Title
P-22	D. Helios	Synergistic anti-cancer effects of Sorafenib and Beauvericin in cervical cancer cell lines
P-23	S. M. Iqbal	Inhibition of hERG Potassium and Cardiac Sodium Channels by BIMU-8
P-24	C. Lampleitner	Pre-clinical screening of novel biodegradable biomaterials for bone tissue engineering
P-25	S. Kanz	Effect of hydroxyapatite nanoparticles for regenerative medicine on primary mouse bone cells in vitro
P-26	W. Shabbir	Glycosylation-dependent activation of ENaC by the TNF lectin like domain derived peptide Solnatide
P-27	A. Stadler	Synthesis and in vitro characterization of novel valerenic acid analogues on $GABA_A$ receptors derived from a ligand-based pharmacophore model
P-28	S. Stadler	Mechanisms of Colon Cancer Entry Into Adjacent Stroma
P-29	M. Crkvenčić	Comparative phytochemical analysis of four Globularia spp.
P-30	A. Koutsoulas	Analysis of volatile constituents of Salvia pomifera and S. fruticosa
P-31	S. L. Latikolik	Honokiol derivatives as RXRα modulators
P-32	A. Lubich	Secondary metabolites of the marine Actinomycete Williamsia maris
P-33	H. Rimac	Polyphenol Constituents Responsible for Antiaggregatory Activity of Propolis
P-34	J. Taibon	Combination of a QuEChERS-based extraction protocol with a fast and selective UHPLC-QTOF-MS assay for the detection and quantification of Metarhizium brunneum metabolites from honey samples
P-35	S. Glasl	Justicia secunda VAHL (Acanthaceae): Phytochemical Investigations and α-Glucosidase Inhibition
P-36	L. Wang	Evodiamine Induces Cholesterol Efflux through Inhibiting ABCA1 Protein Degradation
P-37	C. Gatterer	Phenolic metabolites of Eriosema laurentii De Wild.
P-38	D. Wang	Effect of plasma from high- and low- serum unconjugated bilirubin (UCB) individuals on cholesterol efflux
P-39	M. Amiri	A Novel Approach to Azido-substituted Cyclodextrins
P-40	A. Pany	Analysis of nanoparticles in different semi-solid systems
P-41	R. Schuster	Cajanus cajan – a source for PPARγ activators leading to anti-inflammatory and anti-cancerogenic effects
P-42	E. Roblegg	Alcoholic Beverages and Medications: A safety Concern?

ABSTRACTS

Plenary Lectures

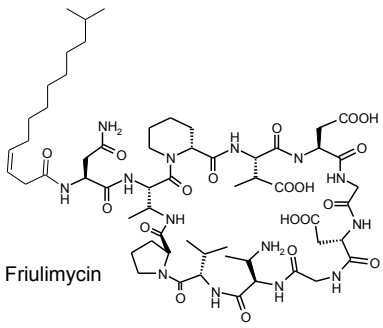
Blast from the past: Will resurrection of under-exploited antibiotics solve the crisis?

Peter Hammann

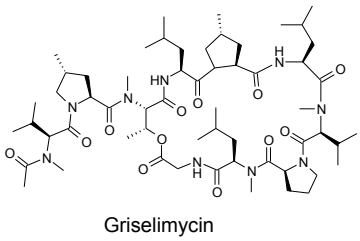
Sanofi-Aventis D GmbH, TSU Infectious Diseases, 65926, Frankfurt, Germany

Despite the fact that resistance of bacterial pathogens is constantly increasing, the number of approved antibacterials is dramatically declining. For example in the early 1980's (1983-87), in a 5 year span, 16 new compounds reached the market, whereas only 3 approvals were observed recently in the same time frame (2008-12). It is even more alarming, that of the 54 compounds approved in the last 30 years, only 2 novel structures were introduced showing only gram positive activity, where as there is a strong need for novel gram negative active antibotics. An analysis of the marketed systemic broad spectrum antibacterial classes today reveals that 17 of the 21 classes are secondary metabolites of micro-organisms. It is noteworthy that 13 structural different antibiotics originating from nature were developed until the end of 1960's, while around 600 antibiotics were described. To date, only 4 more novel classes were marketed, while a total of 3500 antibiotics are known.

Does this confer to the fact that these compounds had no potential for further development, or was the industry just too busy with existing classes not picking up novel ones? Meanwhile, almost all companies involved in antibacterial research started or re-started some projects around theses antibiotics, which were not seriously exploited in the past and indicates that at least the industry believes in their potential. Today a more profound understanding of key parameters influencing pharmacokinetic and pharmacodynamics properties will result in a more directed approach for improving activities and drug properties which could not be done before.



Friulimycin



Griselimycin

Several examples of under-exploited antibiotics, which entered clinical development, will be presented, their status discussed and the lessons learned will be outlined. A special focus will be on the sanofi project Friulimycin [1], a new member of a lipopeptide class with gram positive activity originally isolated in 1951 and Griselimycin [2], a novel cyclopeptide first isolated 1960, with potent activity for *M. tuberculosis*.

1. Vértesy L. E., Ehlers H, Kogler H., Kurz M., Meiwes J., Seibert G., Vogel M. and Hammann P., (2000) *J. Antibiot*. *53*, 816 – 827.

2. Kling A., Lukat P., Almeida D. V., Bauer A., Sordello S., Fontaine E., Zaburannyi N., Herrmann J., Wenzel S. C., König C., Ammerman N. C., Barrio-Perez B., Borchers K., Bordon-Pallier F., Brönstrup M., Courtemanche G., Gerlitz M., Geslin M., Hammann P. E., Heinz D., Hoffmann H., Klieber S., Kohlmann M., Kurz M., Lair C., Matter H., Nuermberger E., Tyagi S.,Grosset J. H.,Fraisse L., Lagrange S., Müller R., (2015) Science, 348, 1106 -1112.

Ocular Drug Delivery: Indispensable or Only Nice to Have?

Achim Göpferich

Department of Pharmacy and Chemistry, University of Regensburg, Universitaetstrasse 31, D-93040 Regensburg, Germany.

A plethora of drugs can be administered in a highly efficient manner to the anterior eye using simple and reliable classical ocular formulations such as eye drops or ointments. In fact, for many years this seemed to be the only way of administering drugs directly to ocular tissues. In recent years, however, spurred by the development of new biologics for drug therapy of the posterior eye, diseases like age related macular degeneration (AMD) and diabetic retinopathy (DR) affecting millions of patients worldwide came into the focus of research and development initiatives. This required a radical change of paradigms since for the therapy of retinal diseases such as AMD, intravitreal injections, formerly considered almost obsolete, became suddenly an indispensable prerequisite. The ocular bioavailability of the developed biologics was too low and side effects too severe to be injected systemically. In the light of these radical changes and due to the potency of drugs administered as simple aqueous intraocular injections, the development of ocular drug delivery systems seemed to be almost completely dispensable.

However, there are a number of problems that may not be overcome with the help of new drugs but only with new approaches of delivering them in a better way. A classical example is glaucoma therapy that suffers not from the lack of potent drugs but from the poor adherence of patients to the therapy [1]. Another example is dry eye disease where we could formulate poorly water soluble drugs as aqueous rather than oily eye drops [2,3]. But also drug therapy of the posterior eye that was highly successful in recent years could profit tremendously from using ocular drug delivery technology. Even tough anti-VEGF antibodies have a tremendous intraocular half-life of several days, their pharmacokinetic properties seem not to be as risk free as it seemed for years. The continuous knockdown of VEGF in the whole retina and not only its disease relevant parts such as blood vessels seems to come with a number of side effects such as geographic atrophy and remodeling of the tissue [4]. Drug delivery systems that delivered such substances more discontinuously could be of tremendous advantage. Likewise the development of new macromolecular therapeutics that offer possibilities of local as well as systemic therapy could be advantageous [5].

The talk will critically evaluate the need of ocular drug delivery technology and give examples where and how they could lead to an improvement of therapies in the future. It will, thereby, be distinguished between technology that could be used in the near future and more experimental approaches that may have implications for future developments [6].

1. Schwartz G.F et al., (2008) *Survey of Ophtalmology*, 53: 57-68.
2. Luschmann C. et al. (2013) *Int. J. Pharm.* 455: 331-337.
3. Luschmann C. et al. (2014) *Europ. J. Pharm. Sci.* 50: 1–8.
4. Grunwald J.E. et al. (2014) *Ophthalmology* 121: 150-161.
5. Pollinger K. et al. (2013) Proc Natl Acad Sci 110, 6115–6120.
6. Goepferich A. (2015) Europ. J. Pharm. Biopharm., doi:10.1016/j.ejpb.2015.05.011.

Aktuelle Herausforderungen in der Krankenhauspharmazie

Silvia Hetz

Arbeitsgemeinschaft Österreichischer Krankenhausapotheker, Spitalgasse 31, A-1091 Wien; Klinikum Wels-Grieskirchen, Krankenhausapotheke Grieskirchnerstraße 41, A-4600 Wels, Österreich

In Österreich gibt es 117 öffentliche Krankenhäuser [1]. Nur 45 verfügen über ein wichtiges Strukturqualitätsmerkmal: Eine eigene Krankenhausapotheke [2]. Dennoch sind Krankenhauspotheker heute stärker gefragt denn je. Aktuelle Herausforderungen im Zusammenhang mit der Arzneimittelversorgung eines Krankenhauses und der pharmazeutischen Betreuung von Patientinnen erfordern Lösungen durch Fachleute, die ihr Augenmerk gezielt auf die Versorgung mit und die optimale Anwendung von Arzneimitteln lenken.

Eine der zentralen Aufgaben, einen hinreichenden Arzneimittelvorrat anzubieten, wird in den letzten Jahren durch Lieferengpässe bei Arzneimitteln bedrohlich erschwert. Als sogenannter "Small Market" mit im europäischen Vergleich eher niedrigen Arzneimittelpreisen, wird Österreich mit Basisarzneimitteln unterversorgt. Knappheiten bei Basisantibiotika, Schmerzmitteln, Zytostatika und Steroiden sind zur alltäglichen Herausforderung für den Krankenhausapotheker geworden.

Ganz anders stellt sich die Situation bei den Hochpreisarzneimitteln dar, für die der "Market Access" in Österreich, anders als in anderen Ländern Europas, praktisch unbeschränkt möglich ist. Die Preisbildung dieser Produkte orientiert sich heute nicht mehr an den Gestehungskosten, sondern viel mehr an dem was der Markt bereit ist zu zahlen. Ein Markt, der im Bereich der Arzneimittel aber nicht wie ein klassischer Markt funktioniert, da Preis und Nachfrage weitestgehend entkoppelt sind.

Die pharmazeutische Industrie produziert in der Regel für große Märkte, für breite Anwendungsmöglichkeiten oder zu astronomischen Preisen – der einzelne Patient, mit seinen speziellen Bedürfnissen, kommt in diesem Konzept nicht vor. Vor diesem Hintergrund ist die Produktion in der Krankenhausapotheke wie eh und je nicht wegzudenken. Gefragt ist die Fähigkeit verschiedenste Arzneiformen herstellen und Rohstoffe verarbeiten zu können. Krankenhausapotheken können sich nicht wie die Industrie auf die Produktion der einträglichsten Arzneimittel beschränken und brauchen deshalb gesetzliche Rahmenbedingungen, die auf diese spezielle Anforderungen Rücksicht nehmen.

Immer mehr Krankenhausabteilungen entdecken in den letzten Jahren ihren Bedarf an klinisch-pharmazeutischer Betreuung. Krankenhausapotheker nehmen an Visiten teil und beraten vor Ort Ärzte, Pflegepersonal und Patienten. Fragen zur richtigen Anwendung, zu Nebenwirkungen und Wechselwirkungen von Arzneimitteln können so auf kurzem Wege geklärt werden. Pharmazeutisches Wissen wird unmittelbar wirksam.

Sei es nun ökonomisches, logistisches oder pharmakologisches *Know-how,* Ärzte und das Krankenhausmanagement gehen selbstverständlich davon aus, dass der pharmazeutische Nachwuchs den jeweiligen Fragen gewachsen ist, die Verantwortung übernehmen kann und von Seite der Universitäten in der notwendigen Anzahl und mit den notwendigen Kompetenzen ausgestattet verfügbar ist.

1. Bundesministerium für Gesundheit (2015) *Krankenanstalten in Zahlen* www.bmg.gv.at
2. Arbeitsgemeinschaft Österreichischer Krankenhausapotheker (2015) www.aahp.at

Harnessing Bacteria for Drug Discovery: from Bioprospecting to Synthetic Biology

Sergey B. Zotchev

Center of Pharmaceutical Sciences, Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.

Although plants and higher fungi have been used by humans for ages as sources for new medicines, drug discovery from bacteria is a relatively new endeavor, its beginning dating back to 1940s. Since then, several decades of bioprospecting in the bacterial kingdom yielded many important anti-microbial and anti-cancer agents (e.g. tetracycline, vancomycin, nystatin, doxorubicin etc), although the rate of discovery declined steeply over the last 20 years. The main reasons for that were high costs of screening coupled to frequent re-discovery of already known bioactive molecules.

It has been demonstrated that certain types of bacteria, namely those belonging to the order *Actinomycetales*, are the most prominent producers of bioactive natural products. Today, although bioprospecting of actinomycetes still remains a viable source of new bioactive compounds [1], approaches based on post-genomic technologies open completely new possibilities for genome-based drug discovery [2]. Actinomycetes' genomes were shown to harbor dozens of orphan gene clusters that are silent under laboratory conditions, but can be activated via genetic engineering. The latter can also be used to alter natural product biosynthetic genes in order to generate new derivatives with improved pharmacological properties [3]. Moreover, Synthetic Biology, a new discipline based on the application of engineering principles to the development of novel biological systems, can also be used to harness bacteria for specific purposes [4].

In this presentation, examples of successful bioprospecting, genome-based elucidation of natural products' biosynthetic pathways and their manipulation, as well as Synthetic Biology-based approaches to engineering actinomycetes for drug discovery and development will be highlighted.

1. Engelhardt K, Degnes KF, Kemmler M, Bredholt H, Fjaervik E, Klinkenberg G, Sletta H, Ellingsen TE, Zotchev SB. (2010) *Appl Environ Microbiol*. 76: 4969-4976.
2. Zotchev SB, Sekurova ON, Katz L. (2012) *Curr Opin Biotechnol*. 23: 941-947.
3. Brautaset T, Sletta H, Nedal A, Borgos SE, Degnes KF, Bakke I, Volokhan O, Sekurova ON, Treshalin ID, Mirchink EP, Dikiy A, Ellingsen TE, Zotchev SB. (2008) *Chem Biol*. 15: 1198-1206.
4. Phelan RM, Sekurova ON, Keasling JD, Zotchev SB. (2015) *ACS Synth Biol*. 4: 393-399

Challenges in Early Drug Discovery and Medicinal Chemistry: Where to Go?

Peter Nussbaumer

Lead Discovery Center GmbH, Otto-Hahn-Strasse 15, 44227 Dortmund, Germany

Early drug discovery (eDD) is within a structural change with a strong trend of industry leaving and academia entering this field. Paradoxically, lack of innovation is among the most cited reasons for the shrinking of the industry pipelines while many academic results are never professionally assessed for their application potential. Academia clearly is a rich source of innovation but how can new basic research concepts find their way into industrial application? Successful direct interactions are extremely rare and would require a substantial cultural change to overcome the differences of the two worlds in language, attitudes, and incentives. A new efficient paradigm for eDD with proven track record is the involvement of a professional translational research center [1]. Such centers function as facilitator and translator at the academia-industry interface and bring the strengths of both worlds together to leverage the high innovation potential of academia by using the robustness and efficiency of industry. The set-up and essential requirements for successful translation will be discussed.

The structural change in the pharmaceutical industry is also affecting medicinal chemists [2]. So far the main education track for comprehensively skilled medicinal chemists, i.e. drug hunters, has been on the job: the best synthetic chemists have been hired by larger pharmaceutical companies and trained internally to medicinal chemists. Who is balancing the "brain drain" and loss of experience with industry having fewer jobs in eDD and who is stepping in with regard to the education and training of future medicinal chemists? Moreover, there are two additional issues in (industrial) medicinal chemistry: 1) combinatorial chemistry, some robust powerful synthetic reactions, and research planning & controlling in industry have nurtured a tendency towards making what is easily feasible rather than the most appropriate compounds and 2) challenges and requirements for medicinal chemists are steadily growing. To ensure sustained quality and achievements, medicinal chemists need to focus on superior design without excluding complex structures a priori, to proactively shape the future of their discipline, to discuss specialization, to intensify exchange between academia and industry, and to remodel education of the next generations of medicinal chemists.

1. Nussbaumer P, Klebl B (2015) *Future Med. Chem.* in press
2. Nussbaumer P (2015) *ChemMedChem* 10: 1133 – 1139.

Rational Cancer Therapy: Fact or Fiction?

Walter Berger

Institute of Cancer Research and Comprehensive Cancer Center, Medical University Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

Cancer is still one of the most frequent causes of human death and successful therapy especially at the disseminated stage a key challenge of modern medicine. Nevertheless, patient survival in many cancer types has recently improved and the rates of cure increased. This is a consequence of several factors including earlier detection, improved local therapy by surgery and radiation but also the development of novel, targeted treatment options at the disseminated stage.

The central problem of systemic cancer therapy lies in the fact that cancer is a disease of our own cells adopting and "abusing" during malignant transformation multiple functions of diverse non-malignant cell types. This relative lack of "foreignness" results in a comparable small therapeutic window with a limited number of potential molecular targets. Consequently, adverse effects are in most forms of systemic cancer therapies key limitations for success. Additionally, based on their genomic instability and, hence, flexibility cancer cells often rapidly activate resistance mechanisms leading to disease recurrence after initially therapy response.

However several important steps for overcoming these limitations have been taken during the last decade. Decoding of the human genome in combination with ultra-high throughput sequencing technologies and systems biology tools recently allowed introduction and development of so called "precision medicine" approaches in oncology. Several targeted compounds have been approved for patient subgroups harboring specific genomic alterations in driver genes and the basis of synthetic lethality in several drug combinations has been elucidated. This led to a completely novel pattern of therapeutic options and stressed the enormous importance of biomarkers for selecting appropriate patient collectives. However, still side effects and resistance development remain as major hurdles.

Recently a novel strategy in immunotherapy of cancer was induced summarized under the term "immune checkpoint inhibition" with - is some cancer types like metastatic melanoma - breathtaking success rates and long term remissions. Background is that cancer cells "abuse" the necessity of our body to limit the duration of immune responses by hijacking immunosuppressive functions of the healthy organism. In this therapeutic approach antibodies are used to block molecular interactions between cancer and immune cells or within different types of immune cells that cause downregulation of the immunresponse so far especially with regard to activated T-cells. Recent studies on the applicability of this approach for multiple cancer types and the development of respective biomarkers will be discussed.

ABSTRACTS

Oral Presentations

PT-1

Prebiotic effect of fructans depending on polymerization degree and structure

Monika Mueller, Helmut Viernstein, Renate Loeppert, Werner Praznik

Center of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna.

Due to their beneficial effect on health, fructans as prebiotics are an important factor in the Functional Food industry [1]. Fructose polymers are accumulated by a great variety of plants including chicory, Jerusalem artichoke or agaves [2, 3]. The influence of structure and polymerization degree (dp) of fructans on the prebiotic potential is not fully elucidated yet. Thus, we compared the prebiotic effect of fructans from chicory, Jerusalem artichoke and agave with different polymerization degree and diverse structures such as inulin-type (only ß2-1 linkages) and mixed-type (combined ß2-1 and ß2-6 linkages with branching) [1- 3].

The influence of the fructan samples on the growth curve of seven probiotic strains was determined including *Lactobacilli casei, paracasei, acidophilus, reuteri or rhamnosus* and *Bifidobacterium animalis.* The method was based on a turbidity measurement and confirmed by conventional cultures on agar plates. Fructans were separated using size exclusion chromatography to obtain fractions with different dp and characterized using ion exchange chromatography. The degradation of fructan oligosaccharides by probiotics was studied using thin layer chromatography.

All fructans showed a significant prebiotic effect on all tested strains with differences depending on the probiotic strain, the fructan type and the dp. Fructan samples with lower dp induced the growth of the probiotics faster than those with higher dp. Branching seems to have a significant influence on bacterial growth kinetics. The correlation between growth promotion and polymerization degree was strain dependent. Some strains grow well with fructans of different dp, some grow with all fructan sources, but dependent on the dp and some grow only with fructans of low dp. Furthermore, the growth is dependent on the degradation process by probiotics. This may be a continuous cleavage of the oligosaccharides to disaccharides and monosaccharides. The exponential phase of growth occurs after most of the oligosaccharides are cleaved to disaccharides and monosaccharides and the growth stops after the sugar source is exhausted. For some strains no degradation was found and thus an uptake by the bacteria and subsequent intracellular cleavage is assumed.

In conclusion, this study contributes to elucidate the influence of structure and dp of the fructans on the prebiotic effect and the dependency of the prebiotic effect and degradation of prebiotics on different strains; both are of high importance for the Functional Food industry and pharmaceutical applications.

1. Peshev D, Van den Ende W. (2014) J Funct Food 8: 348-357.
2. Praznik W, Löppert R, Cruz Rubio JM, Zangger K, Huber A. (2013) Carbohydr Res. 381:64-73.
3. Velázquez-Martínez JR, González-Cervantes RM, Hernández-Gallegos MA, Mendiola RC, Aparicio AR, Ocampo ML. (2014) Molecules. 9: 12660-75.

PT-2

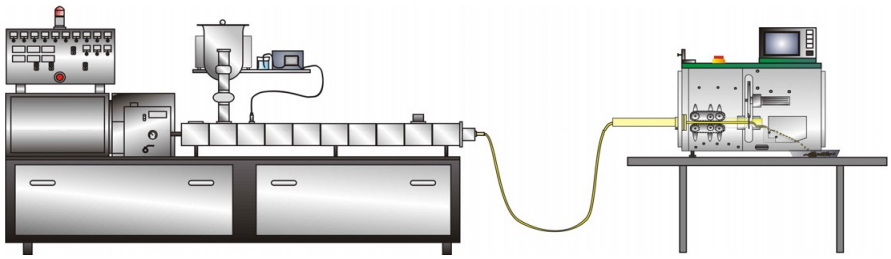
Melt extrusion as a continuous production process for solid dosage forms

Dr. Hubert Rein

Pharmazeutische Technologie der Rheinischen-Friedrich-Wilhelms-Universität
Gerhard-Domagk-Straße 3, D-53121 Bonn-Endenich

Pharmaceutical industry shows an increasing demand for adapting continuous processes in their production lines. Continuous processes provide many benefits in relation to single batch processes: reduction of waste, energy, and finally costs.

Based on a technique, which it is widely used for cutting of cables and wires, a novel manufacturing process for the continuous shaping of hot-melt extruded matrix systems was developed. The extruded strand is cut into biplane tablets by a rotary fly cutter. The process can be monitored by measuring the energy consumption of the motor during the cutting process and the current rotation speed of the fly cutter [1].



Schematic view of the production line; a co-rotating twin-screw extruder (ZSE 27 HP–PH from Leistritz, Nürnberg, Germany) is used for plasticizing the melt. The homogenous strand is processed by a rotary flying-knife cutting machine [2]

Spherical starch pellets were directly and continuously produced using hot-melt extrusion and die-face pelletisation. In contrast to conventional pelletisation procedures, a discontinuous spheronisation step can be avoided. Pellets were produced based on four different starches (corn starch, pea starch, potato starch and waxy corn starch), four different active ingredients (ibuprofen, paracetamol, phenazone and tramadol-HCl) and various additives. The resulting pellets exhibit a large mechanical stability, low porosity and small surface area. Pellets with a very narrow particle size distribution and particle sizes even in the micron scale can be produced [3].

Depending on the used polymers, tablets or pellets with different dissolution characteristics (immediate or prolonged release) can be obtained [4, 5].

1. Thomas Kipping, Hubert Rein, (2013), Eur. J. Pharm. Biopharm, A new method for the continuous production of single dosed controlled release matrix systems based on hot-melt extruded starch: Analysis of relevant process parameters and implementation of an in-process control, May, 84(1):156-71
2. Kudernatsch, (2011), Messebericht TechoPharm 2011, Pharm. Ind., 73, 11, 2056
3. Sebastian Bialleck, Hubert Rein, (2011), Eur. J. Pharm. Biopharm, Preparation of starch-based pellets by hot-melt extrusion, 79, 440-448
4. Sebastian Bialleck, Hubert Rein), (2012), Die Stärke, Drug release mechanisms of hot-melt extruded starch-based pellets, 64, 408–419,
5. Thomas Kipping, Hubert Rein, (2012), J Pharm Drug Deliv Res. Development of Extruded Starch Based Formulations Aimed for Local Drug Delivery to Oral Cavity, Nr. 1, 1

PT-3

Taste Masked Formulations with Advanced Performance

[1]Karin Becker, [2]Diogo Gomes Lopes, [3]Detlev Haack, [4]Michael Stehr, [2]Massimo Bresciani, [2,5]Johannes Khinast, [1]Andreas Zimmer, [2]Sharareh Salar-Behzadi*

1- Pharmaceutical Science, Dep. of Pharmaceutical Technology, Karl-Franzens University, Graz, Austria
2- Research Center Pharmaceutical Engineering (RCPE) GmbH, Graz, Austria; 3- HERMES ARZNEIMITTEL GmbH, Großhesselohe, Germany; 4- CREMER OLEO GmbH & Co. KG, Witten, Germany
5- Technical University of Graz, Graz, Austria

Introduction: An advanced patient-centric strategy for improving patient adherence to medication is the development of "direct to mouth" multi-particulate drug delivery systems, which strike a good and balanced compromise between taste masking and controlled drug release, taking both immediate and extended release into account. Hot-melt coating (HMC) is a suitable technology for manufacturing of such products, offering the advantage of solvent-free processing, leading to significantly shorter processing times and costs. Lipid-based excipients are commonly used with toxicological harmlessness and natural availability at a low price point. The challenge is their polymorphic instability. This abstract presents an advanced predictive approach for development of taste masked controlled release formulations by structuring of lipids and controlling their polymorphic behavior utilizing hot melt technology.

Material and Methods: Two water-soluble active pharmaceutical ingredients (API) were used for coating with mixtures of polysorbate 65 and tripalmitin or pure tristearin, for manufacturing of immediate and controlled release profiles, respectively. A Ventilus® 2.5 fluid bed (Innojet, Germany) with an IHD-1 hot melt device was used for HMC process. The coating quality was evaluated using the dissolution rate and the taste masking in form of a pH-measurement. The *in vitro* pH studies were correlated to the results of the *in vivo* taste masking, to develop a predictive *in vitro* analytical method. The crystallinity and polymorphism were investigated using X-ray diffraction and differential scanning calorimetry (DSC). The release profile at room and accelerated conditions was monitored.

Results and discussion:
Immediate release profile: A polymorphic stable formulation containing tripalmitin and polysorbate 65 was successfully developed, which showed an immediate release profile (85% of release within 30 minutes), an adequate taste masking (pH 3.5 in more than 60 s), polymorphic stability with stable dissolution profile during 6M storage.

Extended release profile: Different inlet temperatures were used for crystallization of lipid to provide unstable α polymorph (A) and stable β polymorph (B) respectively. An additional third structure was created by curing the coating A into β-form in a heating oven with blooming occurring in the inner and outer surfaces of microcapsules (AB). The results showed that the apparent diffusion coefficient of drug was more affected by increasing the tristearin crystallite size and resulting tortuosity, than surface blooming.

Conclusion: We successfully showed the science-based development of stable taste masked multiparticulate formulations with immediate or sustained release profile using lipid based excipients.

In Silico perspective of TRPV1 modulation

Daria Goldmann, Gerhard F. Ecker

University of Vienna, Department of Pharmaceutical Chemistry, Division of Drug Design and Medicinal Chemistry
Althanstrasse 14, 1090-Vienna, Austria

Numerous medicinal chemistry groups have dedicated their efforts to the discovery of agonists and antagonists of the transient receptor potential vanilloid type 1 (TRPV1) as analgesics [1]. Recently determined rat TRPV1 (rTRPV1) structures (PDB IDs: 3J5R, 3J5Q, 3J5P [2-3]) in two distinct conformations allowed us to excel our in silico ligand-based studies on TRPV1 modulation with application of structure-based methods.

First, we constructed a homology model of human TRPV1 (hTRPV1) using available rTRPV1 structure as a template. For the molecular docking experiments we selected two chemical classes of TRPV1 antagonists showing distinct pharmacophoric patterns determined in our recent findings [4]. Next, we analyzed docking poses with protein-ligand interaction fingerprints, common scaffold clustering and pharmacophore modeling. We show that antagonists of two chemical classes adopt different binding modes when bound to TRPV1. However, the most active compounds of both classes form a hydrogen bond with the residue Thr550 and show van der Waals interactions with residues Glu570 and Ile573 which is in agreement with in silico studies by other groups [5-8]. We propose several point amino acid mutations in the binding pocket to experimentally confirm the binding mode hypotheses. In addition, structure-based pharmacophores obtained in this study complement the ligand-based pharmacophore study performed earlier [4]. Finally, we provide the binding hypotheses in the format of a pharmacophore model, which can be implemented for efficient virtual screening of vendor databases for novel TRPV1 antagonists.

We gratefully acknowledge financial support provided by Austrian Science Fund, grant number F3502.

1. Premkumar L.S. AAPS J 2010, 12(3), 361.
2. Cao E. et al. Nature 2013, 504, 113
3. Liao M.et al. Nature 2013, 504, 107.
4. Goldmann D. et al. Future Med Chem 2015, 7(3), 243
5. Voight, E.A. et al. J Med Chem 2014, 57, 7412.
6. Lee J.H. et al. J Comput-Aided Mol Des 2011, 25, 317.
7. Kim M.S. et al. J Med Chem 2012, 55, 8392.
8. Tafesse L. et al. J Med Chem 2014, 57, 6781.

COMP-2

Influence of in vitro pulmonary permeability on in silico prediction of oral inhalation products

Shengqian Wu[a], Annalisa Mercuri[a], Sharareh Salar-Behzadi[a], Massimo Bresciani[a], Amrit Paudel[a], Eleonore Fröhlich[b]

[a] Research Center Pharmaceutical Engineering GmbH, Inffeldgasse 13, 8010 Graz, Austria
[b] Center for Medical Research, Medical University of Graz, Stiftingtalstraße 24, 8010 Graz, Austria

Simulation of pulmonary drug delivery systems is a very complex and challenging process. However, the use of predictive *in silico* models is always a valuable approach during drug formulation development. The current study was designed to investigate the impact of pulmonary permeability obtained from *in vitro* experiments on the *in silico* pharmacokinetic (PK) prediction of three common inhaled APIs from inhalation formulations (formoterol from Foradil® via Aerolizer™, salbutamol from Ventolin® pMDI and budesonide from Pulmicort Turbuhaler®).

In vitro bronchial permeabilities (P_{app}) of formoterol and budesonide were measured experimentally in Calu-3 cells in air-liquid interface (ALI) culture. Bronchial permeability of salbutamol in human primary bronchial epithelial cells in ALI culture was obtained from the literature [1]. All permeabilities were scaled up for the other lung compartments in the simulation. *In silico* pharmacokinetic models of all the formulations were built based on data from literature [2-7] using Gastroplus™ (Simulation Plus, Inc.).

Using experimental pulmonary permeabilitiy data clearly improved the prediction of plasma profiles of formoterol and salbutamol, but did not have any significant impact on the C_{max} of budesonide (Figure A, and B vs C). This trend was most obvious for hydrophilic molecules crossing the epithelium by the paracellular route, such as salbutamol. Peak plasma concentration of budesonide, a compound crossing epithelial barriers predominatly by the transcellular route, was less affected.

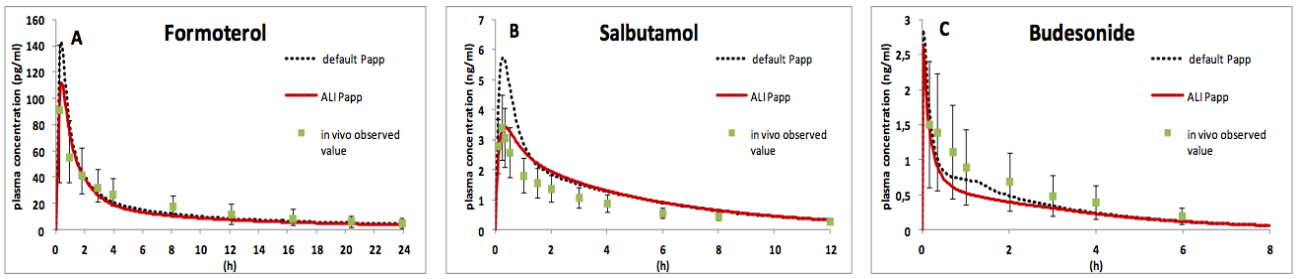


Figure 1. Simulated plasma profile of three APIs from different formulations A) Formoterol via Foradil® Aerolizer™, B) Salbutamol via Ventolin® pMDI, C) Budesonide via Pulmicort Turbuhaler®

Experimental pulmonary permeability data could help to improve the prediction of *in silico* modeling of inhaled APIs using current method, particularly for hydrophilic molecules.

1. Zeng Q, Ge Q, Haghi M, et al.(2014) Eur. Resp. J. 44: 944
2. Thorsson L, Edskäcker S, Conradson T-B (1994) Eur. Resp. J. 7: 1839-44
3. Borgström L, Bondesson E, Moren F et al. (1994)) Eur. Resp. J. 7: 69-73
4. Criée CP, Meyer T, Petro W et al. (2006) J Aerosol Med. 19:466-72
5. Lecaillon JB, Kaiser G, Palmisano M et al. (1999) Eur J Clin Pharmacol. 131-8
6. Xiao-Li D U, Zhu Z H U, Qiang F U et al. (2002) Acta. Pharmacol. Sin. 23: 663-666
7. Barry P W, O´Callaghan C (1997) Eur. Respir. J. 10: 1345-48

PK-1

Personalized Medicine – Compounding of Preparations

Erich Leitner [a], Petra Staubach [b], Holger Reimann [c], Martin Punzengruber [d]

[a]Austrian Chemical Society, Nibelungengasse 11, 1010 Wien, Austria ; [b] Clinical Research Center, Department of Dermatology, Johannes Gutenberg University Medical Center, Langenbeckstrasse 1, 55131Mainz, Germany; [c] Pharmaceutical Laboratory of NRF, Carl Mannich Strasse 26, 65760 Eschborn, Germany; [d] Laboratory of Austrian Chamber of Pharmacists, Spitalgasse 31, 1090 Wien, Austria

Personalized Medicine stands for drugs based on genome analysis by analytical chemistry and use of biologicals. It also fits for conventional drugs specifically prepared for individuals due to exact diagnosis or for the pharmaceutical industry's unmet needs.

Preparations depend on the availability of APIs and excipients as ingredients. They have to obey the rules and requirements in order to prepare. Good analytical tools are sufficient to ensure the quality, however it is always favorable to strive for excellence.

Studies show quality of life is improved for acne patients if they receive topical agents adapted to the acuity of the illness [1]. Atopy ameliorates by lengthening the time between acute episodes if preparations modify or replace manufactured topicals [2].

Conformity to guidelines in the case of anaphylactic emergency, calls for high-dose glucocorticosteroids best available by compounded prednisolone liquid (Prednisolon-Saft 0,1% / 0,5% (m/V) (NRF 34.1.)[3]. It is one of the excellent samples for evidence-based preparations [4].

Up to now, parallelism of the development of pharmaceutical-technical knowledge with medical progress based on empirical data and references has occurred. No real scientific interaction has been observed in this field. Efforts to connect clinical evaluation to the validation of standardized preparations will be the gold standard now and in the future [5]. It should be a basic principle to implement a monograph into the European Pharmacopoeia [6] or one of the International Pharmacopoeias.

In addition, it is necessary to ensure correct and mutual communication on the highest level possible, although medical and pharmaceutical standards do not harmonize best. Because the exchange of technical parameters will not be sufficient, the use of interdisciplinary, accorded guidelines [7] is highly recommended and educational implementation in curricula is necessary.

1. Augustin M, Reich C, Schaefer I, Zschocke I, Rustenbach SJ: Entwicklung und Validierung eines neuen Instrumentes zur Erfassung patientendefinierten Nutzens in der Therapie der Akne. JDDG 6:1-8, 2008
2. [Wohlrab J (ed.): Adjuvante Therapie der Atopischen Dermatitis- Konsenspapier des Arbeitskreises LATOPIA - in: Trends Clin Exp Dermatol, Aachen, Shaker, 2005, vol 4
3. Deutscher Arzneimittel-Codex, Neues Rezeptur- Formularium DAC/NRF 2015/1
4. Staubach P, Anwendungsbeobachtung der NRF-Rezeptur „Prednisolon-Saft" an der Universitäts-Hautklinik Mainz, Symposium der GD-Fachgruppe Magistralrezepturen: Neues zur Qualitätssicherung dermatologischer Rezepturen, Berlin 24.03.2010
5. Staubach, Clinical evaluation J. Skin. Pharmacol. Physiol., 2015, accepted
6. European Pharmacopoeia 8th Edition
7. Leitner E, Staubach P, Personalized medicine by non-licensed preparations - European development from the tradition to the future, 23.WCD, Vancouver 2015
8. Leitlinie der GD Gesellschaft für Dermopharmazie e. V., Dermatologische Rezepturen, 21.03. 2013

PK-2

NF-κB dependent MMP1 expression in MDAMB-231 breast cancer spheroids causes paracrine activation of PAR1 and lymph endothelial barrier disintegration *in vitro*

Chi Huu Nguyen[1,2], Daniel Senfter[2], Jose Basilio[3], Silvio Holzner[2], Serena Stadler[2], Sigurd Krieger[2], Nicole Huttary[2], Daniela Milovanovic[2], Ingrid Simonitsch-Klupp[2], Walter Jäger[1], Rainer de Martin[3], Georg Krupitza[2]

[1]*Department of Pharmaceutical Chemistry, Division of Clinical Pharmacy and Diagnostics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.*
[2]*Clinical Institute of Pathology, Medical University of Vienna, Waehringer Guertel 18-20, Austria.*
[3]*Department of Vascular Biology and Thrombosis Research, Center of Biomolecular Medicine and Pharmacology, Medical University of Vienna, Schwarzspanierstraße 17, A-1090 Vienna, Austria.*

BACKGROUND: Breast cancer is the most common cancer in women spreading mainly via lymphatics [1]. We investigated the role of NF-kB during tumour extravasation through the endothelial barrier, which is a crucial step of metastasis.

METHODS: *In vitro* extravasation was studied by a robust three-dimensional cell culture assay consisting of MDA-MB231 breast cancer cell spheroids placed on top (apical side) of lymph endothelial cell (LECs) monolayers. As quantitative readout, tumour spheroids repel LECs thereby generating disintegrated areas in the LEC monolayer, so call “circular chemorepellent induced defects” (CCIDs), through which tumour emboli extravasate lymphatics [2]. Si-RNAs and pharmacological inhibitors were used to block individual members of the NF-kB family as well as downstream targets of NF-kB in LECs and in MDA-MB231 breast cancer spheroids. The downstream mechanisms were furthermore studied by western blotting analysis, q-PCR and intracellular calcium assay.

RESULTS: Canonical NF-kB members (RELA, NFKB1 and NEMO) inhibited CCIDs by ~ 20-30%, whereas non-canonical NFkB member (RELB, NFKB2 and NIK) inhibited CCIDs by only ~ 10-15%. RELA and NFKB1 regulated MMP1 expression in MDA-MB231 cells, which in turn activated PAR1 in adjacent LECs. Knock-down of MMP1 in MDA-MB231 spheroids as well as inhibition of PAR1 in LECs reduced CCIDs formation by ~ 30 %. Recombinant MMP1 induced intracellular Ca^{2+} release in LECs, which was suppressed by PAR1 antagonist SCH79797. Furthermore, MMP1 induced PAR1-dependent phosphorylation of MLC2 and FAK in LECs, which is necessary for directional cell migration such as observed during CCID formation.

CONCLUSION: The data demonstrate a functional intercellular axis: RELA/NFKB1 – MMP1 (in MDA-MB231) – PAR1- Ca^{2+}, p-MLC2, p-FAK (in LEC) in a validated model resembling breaching of the endothelium by tumour emboli and link inflammation to cancer progression. This provides potential targets and strategies for pharmacological intervention.

References

1. Vonach C, Viola K, Giessrigl B *et al* (2011). Br J Cancer 105: 263-271.
2. Kerjaschki D, Bago-Horvath Z, Rudas M *et al* (2011). J Clin Invest 121: 2000-2012

hERG channel inhibiting alkaloids from *Nelumbo nucifera*: How safe is the "Lotus Leaf Diet"?

Ulrike Grienke[a], Christina E. Mair[a], Priyanka Saxena[b], Igor Baburin[b], Olaf Scheel[c], Markus Ganzera[d], Daniela Schuster[e], Steffen Hering[b], Judith M. Rollinger[a]

[a] Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria; [b] Department of Pharmacology and Toxicology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria; [c] Cytocentrics Bioscience GmbH, Tannenweg 22k, 18059 Rostock, Germany; [d] Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria; [e] Institute of Pharmacy/Pharmaceutical Chemistry, Computer-Aided Molecular Design Group, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria

The human Ether-a-go-go Related Gene (hERG) potassium channel is responsible for sending an electrical current across the cell membrane and plays a key role during the repolarisation phase of the cardiac action potential. An interference or blockage of this ion channel can result in ventricular tachyarrhythmia, a long QT syndrome, and in an increased incidence of sudden deaths [1].

In the "hERGscreen" project, the aim is to identify hERG blockers by screening extracts or fractions of commonly consumed herbal materials in a fast and reliable *in vitro* assay on *Xenopus* oocytes [2].

Here, supported by virtual predictions of a recently generated pharmacophore model [3], an alkaloid fraction of *Nelumbo nucifera* Gaertn. (lotus) leaves revealed 50% hERG channel inhibition at 100 µg/mL. Lotus leaves are widely advertised as herbal supplements to achieve weight loss without any side effects. Currently, this is known as the "lotus leave diet".

To further characterize the hERG blocking activity of *N. nucifera* alkaloids, separation was performed via liquid liquid chromatography using fast centrifugal partition chromatography (FCPC). As a result, four aporphine-type alkaloids, i.e. nuciferine (1), (-)-asimilobine (2), O-nornuciferine (3), and N-nornuciferine (4), and one isoquinoline-type alkaloid, i.e. liensinine (5) were isolated and structurally identified. At 100 µM, compounds 1, 3, and 4 revealed distinct hERG blocking activities in the range of 45 to 80%. These constituents were further studied on hERG channels stably expressed in human embryonic kidney (HEK293) cells. Compounds 1, 3, and 4 induced a concentration-dependent decrease in current amplitude according to the voltage steps and tail currents of hERG with IC_{50}s of 2.89, 7.91, and 9.75 µM [4].

This study provides insight into predicted ligand-target interactions and phytochemical analyses of respective materials which underline the high relevance of these findings concerning a possible restriction in the use of lotus herbal supplements as dietary products.

Supported by EU-FP7-PEOPLE-ISRES Marie Curie: hERGscreen, 295174

1. Sanguinetti MC, Tristani-Firouzi M (2006) Nature 440, 463-469.
2. Schramm A, Baburin I, Hering S, Hamburger M (2011) Planta Med. 77, 692-677.
3. Kratz JM, Schuster D, Edtbauer M, Saxena P, Mair CE, Kirchebner J, Matuszczak B, Baburin I, Hering S, Rollinger JM (2014) J. Chem. Inf. Model. 54 (10): 2887-2901.
4. Grienke U, Mair CE, Saxena P, Baburin I, Scheel O, Ganzera M, Schuster D, Hering S, Rollinger JM (2015) J. Agric. Food Chem. 63 (23): 5634-5639.

PKN-2

In vivo characterisation of voacangine - a hERG blocking iboga alkaloid from *Voacanga africana*

Christina E. Mair[a], Carolina M. Silva[b], Ulrike Grienke[a], Fernando Carreño[b], Jadel Müller Kratz[c], Teresa Dalla Costa[b], Judith M. Rollinger[a]

[a] Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [b] Faculty of Pharmacy, Federal University of Rio Grande do Sul, Av. Ipiranga, 2752, Porto Alegre CEP 90610-000, RS, Porto Alegre, Brazil; [c] Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina, 88.040-900 Florianópolis, Santa Catarina, Brazil

The bark of the tropical tree *Voacanga africana* Stapf ex Scott Elliot (*Apocynaceae*) and related species is commonly used in West and Central African folk medicine. Recently, voacangine (1), the major iboga-type alkaloid contained in *V. africana* bark, was found to be a potent hERG channel blocker which might imply possible cardiotoxicity [1]. In contrast to its derivative ibogaine little is known about pharmacokinetic characteristics of voacangine. Since this plant material is becoming increasingly popular as legal high in Europe, the aim of this study was to assess the pharmacokinetics of 1. It was given as pure compound and in an ethanolic extract of *V. africana* bark, to allow for a critical discussion of its potential cardiotoxic risk *in vivo*.

A precise and sensitive LC-MS/MS method was developed and validated according to FDA guidelines to detect 1 in rat plasma. Independent of the investigated drug concentration, 1 showed a high plasma protein binding of 98.7±0.29%. Pharmacokinetics were investigated by comparing four groups (G1-G4, n=5-7/group). G1 received a single 5 mg/kg *i.v. bolus* dose of 1; G2 and G3 received single 25 mg/kg or 50 mg/kg doses of 1 *p.o.*, respectively; G4 received a single 500 mg/kg *p.o. bolus* of the ethanolic *V. africana* bark extract, containing 9.71% of 1. Non compartmental analysis showed a volume of distribution (Vz) of 6.1 ± 3.4 L/kg for the *i.v.* dosing, a clearance (CL) of 1.4±1 L/h/kg, and an average half-life ($T_{1/2}$) of 6.0±2.0 h. The oral doses of 1 indicated linear pharmacokinetics since no statistical differences were observed in CL, Vz and $T_{1/2}$ compared to the *i.v.* dose. Oral bioavailability (F) was ~12% for both doses and 8.0% for 1 in the extract.

These findings provide valuable data to estimate the risk for cardiotoxic events provoked by consumption of the hERG blocking natural product 1.

Supported by EU-FP7-PEOPLE-ISRES Marie Curie: hERGscreen, 295174

1 Kratz JM, Schuster D, Edtbauer M, Saxena P, Mair CE, Kirchebner J, Matuszczak B, Baburin I, Hering S, Rollinger JM (2014) J. Chem. Inf. Model. 54 (10): 2887-2901.

PC-1

A few Remarks on the Quality of Published Carbon-NMR Data

Norbert Haider[a], Wolfgang Robien[b]

[a] *Department of Pharmaceutical Chemistry, University of Vienna, Althanstraße 14, A-1090 Vienna*
[b] *Department of Organic Chemistry, University of Vienna, Währinger Straße 38, A-1090 Vienna*

The structure elucidation of natural products and synthetically available pharmaceuticals is done predominantly by means of different spectroscopic techniques. Among these methods carbon NMR-spectroscopy contributes a significant amount of information, because it allows direct insight into the molecular skeleton. Furthermore the prediction of carbon chemical shift values for a given structural proposal can be done with high precision (usually within 1% of the typical chemical shift range) using different computational models ranging from DFT to database-oriented techniques.

Disease pattern: Based on the evaluation of some 500,000 published NMR-spectra from the public-domain literature an error rate of approximately 8% has been detected [1,2] – these errors are either assignment errors and/or wrong structural proposals. Many assignments are done by comparison with suitable reference material instead of applying state-of-the-art 2D-NMR techniques therefore propagating existing assignment errors into new compounds. As a consequence of this strategy these errors become more difficult to be detected because of their better statistical robustness.

Therapy: A WEB-Service named 'CSEARCH-Robot-Referee' [3] has been developed which allows a very detailled compatibility check between a given structure and the peaklist (either assigned or unassigned shift values with multiplicity information as an option). The result from this 'Robot-Referee' is similar to the classification scheme in the peer-reviewing process proposing either „Accept as it is“, „Minor Revision“, „Major Revision“ or „Reject“. In case that the evaluation process recommends a „Major Revision“ or „Reject“ a similarity search using the given peaklist is automatically launched over 65 millions of predicted CNMR-spectra of the PUBCHEM-structures giving a ranked list of alternate proposals.

Contraindication: The systematic application of the technology avoids wasting of ressources into already known compounds, furthermore bizarre structure proposals could be avoided to be published in high-impact journals maybe leading to a somewhat smaller list of publications in the own curriculum.

Dosage: The CSEARCH-Robot-Referee is available for free after initial registration and should be used frequently to verify the result of the structure elucidation process. This workflow has been already integrated into the publication process of the „European Journal of Organic Chemistry“ [4] - a high-impact journal from Wiley. In the upcoming release of Bruker's TOPSPIN/CMC-se program suite [5] direct access to this service will also be possible.

Application: The lecture will give a short overview on strange assignments, bizarre structure proposals including better alternatives – every example taken from the public-domain literature will be accompanied by the result of the automatic evaluation showing how easily such errors can be avoided by application of state-of-the-art software technologies.

1. Robien W. TrAC, 27,914(2009) DOI:10.1016/j.trac.2009.03.012
2. Application note from ACD; http://www.acdlabs.com
3. http://nmrpredict.orc.univie.ac.at/c13robot/robot.php
4. http://www.eurjoc.org DOI: 10.1002/ejoc.201403496
5. https://www.bruker.com/products/mr/nmr/nmr-software/software/topspin/overview.html

PC-2

Modern Practice for Material Identification and Quality Control in Pharmacies and Pharmaceutical Industry

Heinrich Orsini-Rosenberg[a], Renate Dietl[a], Domenico Martoccia[b], Armin Gembus[c]

[a]*Bruker Austria GmbH, Lemböckgasse 47b, A-1230 Vienna, Austria*
[b]*Bruker Optics GmbH, Industriestrasse 26, CH-Fällanden, Switzerland*
[c]*Bruker Optik GmbH, Rudolf-Plank-Str. 27, D-76275 Ettlingen, Germany*

Techniques of vibrational spectroscopy like FT-IR, FT-NIR and Raman spectroscopy are the methods of choice for raw material identification and quality control due to their high selectivity. Each of these methods have advantages and disadvantages concerning their application in the pharmaceutical fields.

Technological developments in instrumentation during the past years have led to miniaturization and increase of ruggedness, so that the application of FTIR-ATR spectroscopy has been widely spread. It is now even available for pharmacies for raw material identification and quality control of traditional formulations.

For FT-IR, typically a spectra comparison against a single reference spectrum is performed. The high selectivity of the sharp mid-IR bands provide enough information in order to uniquely identify a given substance. With FT-NIR, the less specific spectra have to be analysed by more elaborate chemometric techniques – however the near-IR spectral range provides other advantages like online process monitoring with long fiber probes, or the analysis of large amounts of sample material.

Lastly, Raman spectroscopy is as specific as mid-IR, and even enables measurements through polymeric or vitreous containers. But in many cases material verification is prevented by fluorescence.

Recent innovations in the design of handheld Raman spectrometers like Sequentially Shifted Excitation SSETM technique [1] [2] [3] manages an effective mitigation of fluorescence, and therefore provide higher performance for substantially increased accessibility to many material systems.

1. J.B. Cooper, M. Abdelkader, K.L. Wise, Appl. Spectros. 67, 973–984 (2013).
2. J.B. Cooper, S. Marshall, R. Jones, M. Abdelkader, K.L. Wise, Appl. Optics. 53, 3333–3340 (2014).
3. US patent 8,570,507 B1

An Open PHACTS Knime workflow to collect compound data from public databases

Daniela Digles, Gerhard F. Ecker

University of Vienna, Department of Pharmaceutical Chemistry, Althanstraße 14, 1090 Vienna, Austria.

The Open PHACTS project [1] integrates several public databases, thus allowing answering questions relevant for research in the drug-discovery process [2]. The data collected in the project can be accessed with web tools, such as the Open PHACTS Explorer (www.openphacts.org/explorer) or by using the programming interface (API, available from dev.openphacts.org). To answer questions requiring several different queries in an automated way, workflow tools such as KNIME or Pipeline Pilot are a valuable resource. Such KNIME workflows were used for several use-cases [3] and to provide answers to drug-discovery research questions [4].

In the current work, a workflow to easily generate a summary of known data connected to a compound of interest is presented. The workflow was generated using KNIME, with the Open PHACTS nodes available from https://github.com/openphacts/OPS-Knime.

Starting from the depiction of a molecule, similar molecules are retrieved. Information collected directly for the molecule includes function and toxicity annotations (from Drugbank), the role of the molecule (from ChEBI), and biological pathways containing this molecule (from Wikipathways). In the next step, proteins where the molecule is reported to be active in ChEMBL are returned, and the connection of the proteins to biological pathways (from Wikipathways) and to diseases (from DisGeNet) are shown. The data from all these sources was retrieved via the Open PHACTS API, easily connecting the identifiers used in the different databases. The finished workflow can be easily adapted to query for a different molecule, and can be executed without knowledge about programming.

The workflow allows the preparation of a first overview on data known for a potential compound of interest, including data for similar compounds. Links to the original data sources are retained, allowing manual curation of the collected associations.

Acknowledgements: The work has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no. [115191], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and in-kind contribution of EFPIA companies.

1. Williams A J, Harland L, Groth P, *et al.* (2012) Drug Discov. Today 17: 1188 – 1198.
2. Azzaoui K, Jacoby E, Senger S, *et al.* (2013) Drug Discov. Today 18: 843 – 852.
3. Ratnam J, Zdrazil B, Digles D, *et al.* (2014) PloS One: e115460. doi: 10.1371/journal.pone.0115460.
4. Chichester C, Digles D, Siebes R, *et al.* (2015) Drug Discov. Today 20: 399 – 405.

PC-4

Development and preclinical characterization of partial farnesoid X receptor agonists for metabolic disorders

Daniel Merk

Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany; merk@pharmchem.uni-frankfurt.de

As a member of the nuclear receptor superfamily, farnesoid X receptor (FXR) acts as cellular regulator of bile acid homeostasis.[1] In this role, FXR controls the expression of several genes involved in bile acid, lipid and glucose homeostasis.[2-4] The involvement of FXR in several metabolic pathways offers a novel attractive drug target for the treatment of metabolic disorders, i.e. dyslipidemias and diabetes mellitus and first beneficial effects of pharmacological FXR activation on hyperglycemia have yet been reported from the clinical development of obeticholic acid which is the most developed FXR agonist so far.[5] However, the orchestra of nuclear receptors and their target genes is a very complex network that also bears the risk of severe side-effects as it has e.g. been observed for the glitazones that are agonists on the peroxisome proliferator-activated receptor γ (PPARγ). A potential way to reduce the risk of such undesired effects of nuclear receptor over-activation is the development of partial agonists that only activate the respective receptor to moderate amplitude.[6]

Starting from a virtual screening program, we have developed a set of highly potent FXR partial agonists by systematic structure-activity-relationship studies and repeated rounds of structural optimization.[7-9] Preparation of co-crystal structures of selected representatives helped the structural optimization process and improved our knowledge on the molecular mechanism of partial FXR activation.

The resulting agents are selective for FXR, display EC50 values for partial FXR activation in the low nanomolar range and activate the receptor to an amplitude of around 40% compared to the physiological agonist chenodeoxy cholic acid. Intensive in vitro pharmacological characterization revealed a direct interaction with the FXR-LBD and partial agonistic potency on all investigated FXR target genes. Moreover, the compounds showed good metabolic stability and low toxicity.[7-9] First in vivo data is very promising and warrants further exploration of the strategy and the compound class. The current optimization addresses the improvement of drug-likeness, stability and bioavailability to obtain a further optimized model agent for intensive in vivo investigation of partial FXR agonism as novel strategy to treat metabolic disorders.

1. Makishima, M. et al.: Science 1999, 284(5418): 1362–1365.
2. Kuipers, F., Bloks, V., Groen, A.: Nat. Rev. Endocrinology 2014, 10(8): 488–498.
3. Düfer, M. et al.: Diabetes 2012, 61(6): 1479–1489.
4. Fang, S. et al.: Nat. Med. 2015, 21(2): 159–165.
5. Mudaliar, S. et al.: Gastroenterology 2013, 145(3): 574–582.
6. Merk, D., Steinhilber, D., Schubert-Zsilavecz, M.: Future Med. Chem. 2012, 4(8): 1015–1036.
7. Merk, D. et al.: Bioorg. Med. Chem. 2014, 22(8): 2447–60.
8. Merk, D. et al.: J. Med. Chem. 2014, 57(19): 8035–8055.
9. Merk, D. et al.: Bioorg. Med. Chem. 2015, 23(3): 499–514 and unpublished data

PC-5

Synthesis and enantioseparation of eight new amphetamine derivatives by indirect gas chromatography-mass spectrometry after derivatization with (R)-(+)-α–methoxy-α-trifluoromethylphenylacetic acid (MTPA) or with (R)-(-) menthylchloroformate or by HPLC-UV

Jennifer A. Weiß, Kian Kadkhodaei, Martin G. Schmid*

Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, University of Graz, Universitaetsplatz 1, A-8010 Graz, Austria

New amphetamine derivatives and cathinones gained high popularity on the illegal drug market. They are distributed under the synonym “legal highs” and frequently consumed, because they are cheaper and easier available than other popular illicit drugs. These amphetamine type stimulants possess a powerful central nervous system stimulation ability.

In the last years, a broad spectrum of new psychoactive drugs appeared on the drug market to circumvent laws, which regulate consumption and sale of controlled substances. Among others, many of these new psychoactive compounds show amphetamine or cathinone structure and a GC-MS library was generated with compounds purchased via the Internet. Since reference standards are very cost intensive and sometimes unavailable, the aim of this study was to synthesize eight new amphetamine derivatives.

2-Fluoroamphetamine (2-FA), 2-fluoromethamphetamine (2-FMA), 4-bromoamphetamine (4-BA), 4-bromomethamphetamine (4-BMA), 4-nitroamphetamine (4-NA), 4-nitromethamphetamine (4-NMA), 2-chloroamphetamine (2-CA) and 2-chloromethamphetamine (2-CMA) were synthesized in microscale amounts and investigated concerning their identity and their enantiomeric status either by GC-MS or by HPLC.

For GC-MS analysis the compounds were derivatized with (R)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) and (R)-(-)menthylchloroformate, as two different chiral derivatization agents. With this indirect chiral separation method there is no need for an expensive chiral stationary phase. After derivatization with MTPA it was feasible to resolve eight compounds out of ten successfully, achieving resolution factors ranging from 1.68 for 4-BMA to 6.45 for 2-CA, respectively. Less satisfying resolution factors were obtained after derivatization with (R)-(-)menthylchloroformat.

Chiral separation by HPLC was carried out using a Lichrospher RP-18e column and sulphated beta-cyclodextrin as chiral mobile phase additive.

With this method, successful enantioresolution of eight compounds was shown as well.

COMP-3

Towards a structural understanding of Cantú disease associated gating perturbations in the K_{ATP} potassium channel

Eva-Maria Plessl-Zangerl, Anna Stary-Weinzinger

Department for Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Cantú syndrome is a rare disease affecting a small number of people worldwide. It is characterized by multiple symptoms including distinctive facial features, cardiac abnormalities, hypertrichosis and lymphedema. This genetic disorder is caused by dominant gain-of-function mutations in the ATP-dependent potassium channel K_{ATP} impeding channel closure at the correct ATP concentration. These channels consist of a sulfonylurea transporter subunit (encoded by the ABCC9 gene), and the pore-forming $K_{IR}6.1$ subunit (encoded by the KCNJ8 gene).

To obtain insights into the structure of this large protein complex, homology modeling techniques are applied. For the $K_{IR}6.1$ subunit we used the crystal structure of $K_{IR}3.2$ as template (pdb-code 3SYA; ~50% sequence identity). The sulfonyl urea transporter subunit was modeled on the distantly related TM287/288 ATP binding cassette transporter (pdb-code 4Q4H; ~25% sequence identity).

These models are currently used to establish the structural basis of known Cantú associated mutants. Figure 1 shows two gain-of function mutations (V65M and C176S), located in the pore module of the K_{ATP} channel. Both amino acids are in close contact in the closed channel state. During channel opening, this region undergoes large conformational changes, including rearrangements of these two amino acids. To further investigate how perturbations in this area influence gating, we are currently performing molecular dynamics simulations.

From these studies, a better understanding of Cantú disease and the function of the K_{ATP} channel in general (a well-known pharmaceutical target for treatment of diabetes mellitus) are expected.

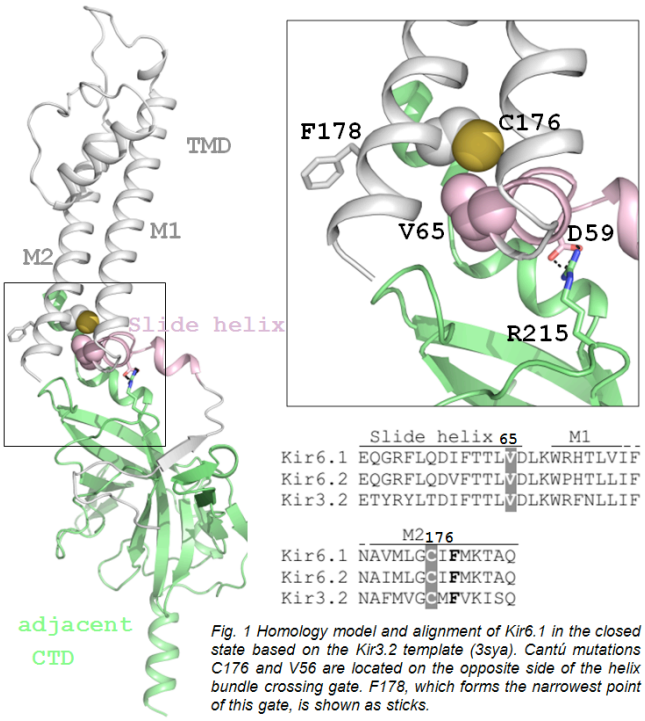


Fig. 1 Homology model and alignment of Kir6.1 in the closed state based on the Kir3.2 template (3sya). Cantú mutations C176 and V56 are located on the opposite side of the helix bundle crossing gate. F178, which forms the narrowest point of this gate, is shown as sticks.

POSTER ABSTRACTS

P-1

A molecular dynamics approach to study P-glycoprotein in complex with propafenone-type ligands

Melanie Grandits[a], Gerhard F. Ecker[a]

[a]*Pharmacoinformatics Research Group, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*

P-glycoprotein (Pgp) plays a role in multidrug resistance by showing higher expression levels in resistant cells. This ABC-transporter binds substrates from the membrane and causes an immediate drug efflux, which is a major drawback for therapeutics [1-4].
The aim of this study is to get insight into the different affinities of the protein for various propafenone-type ligands as shown experimentally [5]. To achieve this, a molecular dynamics (MD) approach is used to determine the interaction pattern, the binding free energy and further the kinetics to understand their influence on the selectivity profile.
In this study a homology model of the human Pgp was generated based on a refined murine structure [6,7]. The apo protein embedded in a membrane was simulated to obtain a stable structure and clustering was used to filter out diverse structures to use them as starting points for subsequent simulations of the complex. The complexes were generated by positioning the ligands in multiple orientations at the entry of the transmembrane region. This allows the ligands to freely diffuse to the binding site. The binding free energies were determined by using the Linear Interaction Energy [8] approach and a Markov state Model [9] will be constructed to determine the kinetics.
Results show a different binding pattern between the ligands, revealing residues already indicated in previous docking [5] and MD studies [10]. A difference in the binding free energy of 9 $kJ \cdot mol^{-1}$ was found, which is in agreement with experimental data.
In summary, a difference between the ligands could be observed in terms of binding position, interactions as well as binding free energy. This gives a first understanding of their influence on the selectivity profile, which will further be supported by assessing the contribution of the kinetics.

We gratefully acknowledge financial support provided by the Austrian Science Fund, grant F3502 (SFB35).

References:
1. Borst P, Evers R, Kool M, Wijnholds J (1999) Biochim Biophys Acta 1461: 347–357.
2. *Glavinas H, Krajcsi P, Cserepes J, Sarkadi B (2004) Curr Drug Delivery 1: 27–42.*
3. *Sharom FJ (2008) Pharmacogenomics J 9: 105–127.*
4. Sharom FJ (1997) J Membr Biol 160: 161-175.
5. Klepsch F, Chiba P, Ecker GF (2011) PLoS Comp Biol 7: e1002036.
6. Li J, Jaimes KF, Aller SG (2014) Protein Sci 23: 34- 46.
7. Klepsch F, Ecker GF (2010) Mol Inform 29: 276-286.
8. Aqvist J, Medina C, Samuelsson JE (1994) Protein Eng 7: 385–391.
9. Pande VS, Beauchamp K, Bowman GR (2010) Methods 52: 99-105.
10. Subramanian N, Condic-Jurkic K, Mark AE, O'Mara ML (2015) J Chem Inf Model

P-2

Elucidation of the structure of a strigolactone ABC-transporter in Petunia hybrida

Eva Hellsberg, Floriane Montanari, Gerhard F. Ecker

University of Vienna, Department of Pharmaceutical Chemistry, Althanstrasse 14, 1090 Wien

The knowledge about phytohormones and their transport is growing steadily and there is a need for an understanding of the molecular basis of substrate and inhibitor interaction. The class of strigolactones is one of the current centers of attention. They have several roles as stimulation and recognition signals in plants, but the knowledge about their regulating function in shoot branching is quite new. In 2012, the ABC transporter PDR1 in *Petunia hybrida* (PhPDR1) was identified as a strigolactone transporter [1]. The PDR proteins belong to the ABCG family, which exhibits a reverse topology to the other ABC members.

The scientific aim of this project is to elucidate the molecular 3D structure of PhPDR1. At first, we performed a comprehensive investigation to define the transmembrane domains, their containing helices and their locations, which was a challenging task according to the little information yet known. We combined the results extracted from prediction tools, multiple sequence alignments and information from literature to draw the determining conclusions.
The final choice of the template was based on a multiple sequence alignment with the potential templates, including crystallized ABC transporters reported in the PDB and the high-quality, reliably validated homology model of PDR5 in *Saccharomyces cerevisiae* [2]. The latter was already used as a template to model the 3D structure of Cdr1 in *Candida albicans* [3]. PDR5 shows the highest sequence identity percentage and thus was chosen as template for further comparative modelling steps.
We created the homology models with the modeller software [4] and took the decision for a final model based on several assessment methods.
In the validation process, we examined the polar residues of the transmembrane helices and the electrostatic potentials of the model to characterize the translocation chamber.
Finally, we conducted a small docking study, where we docked the strigolactone orobanchol (which is evidently transported by PhPDR1) into our model and clustered the results into several groups. This led to first ideas of binding poses and amino acids which could be involved in the binding mode.

1. Kretzschmar T, Kohlen W, Sasse J, Borghi L, Schlegel M, Bachelier JB, Reinhardt D, Bours R, Bouwmeester HJ, Martinoia E. A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. Nature 2012; 483: 341–344.
2. Rutledge RM, Esser L, Ma J, Xia D. Toward understanding the mechanism of action of the yeast multidrug resistance transporter Pdr5p: A molecular modeling study. J Struct Biol 2011; 173: 333–344.
3. Rawal MK, Khan MF, Kapoor K, Goyal N, Sen S, Saxena AK, Lynn AM, Tyndall JDA, Monk BC, Cannon RD, Komath SS, Prasad R. Insight into Pleiotropic Drug Resistance ATP-binding Cassette Pump Drug Transport through Mutagenesis of Cdr1p Transmembrane Domains. J Biol Chem 2013; 288: 24480–24493.
4. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen M-Y, Pieper U, Sali A. Comparative protein structure modeling using Modeller. Curr Protoc Bioinforma Ed Board Andreas Baxevanis Al 2006; Chapter 5: Unit 5.6.

P-3

Structure based classification for BSEP/ABCB11 Inhibitors using comparative structural modeling of human BSEP

Sankalp Jain, Gerhard F.Ecker

University of Vienna, Department of Pharmaceutical Chemistry, Althanstrasse 14, 1090 Vienna, Austria

ABCB11, also known as Bile Salt Export Pump (BSEP), is expressed in the bile canalicular membrane where it transports conjugated monovalent bile acids from the hepatocytes into the bile[1]. BSEP can be inhibited by a number of drugs, causing decreased bile flow which leads to the accumulation of cytotoxic bile salts in the liver, and thus resulting in drug-induced cholestasis or liver injury[2]. The aim is to carry out structure based studies for BSEP and to extract information about the characteristics of inhibitors and non-inhibitors, in order to better understand their inhibitory mechanism.

Using the FASTA sequence of human BSEP (UNIPROT ID: O95342) as a query, a position-specific iterative BLAST (PSI-BLAST) search was performed against the Protein Data Bank. The most structurally related template protein (corrected mouse P-glycoprotein structure PDB ID: 4M1M) was selected based on sequence identity and atomic resolution. Multiple homology models were constructed using MODELLER 9.13 and Maestro. Energy minimized models were then evaluated using DOPE Score and G-Factor and the best model was selected using Ramachandran plot and Q-score. Prediction of potential ligand-binding site was performed using SiteFinder as well as SiteMap to allow subsequent flexible docking studies of a series of inhibitors of BSEP.

For the best model, we received a normalized DOPE score of -0.513, and Qmean of 0.599 with 91.6% residues in the allowed region. Docking of a set of inhibitors and non-inhibitors (using ChemScore scoring function), allowed a structure-based classification with an accuracy of 81% on the training set (113 inhibitors and 295 non-inhibitors) and 70% on an external test set comprising 53 inhibitors and 131 non-inhibitors.

The structure-assisted docking models show reasonably good prediction accuracy and therefore, a workflow comprising prescreening with simple descriptors, classification by machine learning techniques and postprocessing by structure-based methods would provide accurate prediction with information useful for further drug development.

We acknowledge financial support provided by the Austrian Science Fund, grant SFB35, and by the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115002 (eTOX).

1. X. Cheng, D. Buckley, and C. D. Klaassen, “Regulation of hepatic bile acid transporters Ntcp and Bsep expression,” *Biochem. Pharmacol.*, vol. 74, no. 11, pp. 1665–1676, Dec. 2007.
2. S. Amer and A. Hajira, “A Comprehensive Review of Progressive Familial Intrahepatic Cholestasis (PFIC): Genetic Disorders of Hepatocanalicular Transporters,” *Gastroenterol. Res.*, vol. 7, no. 2, pp. 39–43, May 2014.

Design and synthesis of new diethylstilbestrol derivatives as anti-breast cancer agents

Alexandra Knox, Ronald Gust

CCB – Centrum für Chemie und Biomedizin, Institute of Pharmacy, Department of Pharmaceutical Chemistry, University of Innsbruck, Innrain 80-82, A-6020 Innsbruck, Austria

The estrogen receptor (ER) is a member of the group of nuclear receptors and target of estrogenic hormones. It can be found ubiquitous in the human body such as in many reproductive tissues (mammary gland, uterus) the skeletal and cardiovascular system and also specific parts of the brain [1].
Regulation of the estrogen receptor activity can be achieved via different ways. The target predominantly chosen for ER-antagonists is the ligand binding domain (LBD) [2]. The resulting ER antagonistic effect on breast cells by the stilbene derivative tamoxifen is one of the main therapy strategies in mammary carcinoma treatment [3].
However, in the last few years, inhibitors of the coactivator binding have been developed to achieve a new targeting of ER-alpha.
Previously, ER-beta was crystallized with two 4-hydroxytamoxifen (4-OHT) molecules.
4-OHT is bound in the LBD, as well as at the hydrophobic surface of the coactivator binding groove [4]. Docking studies using the program GOLD demonstrated that diethylstilbestrol (DES) - another stilbene derivative - interacts in a similar way at these sites. With this information and previous results of Shan et al. [5], DES derivatives with a hexanoic acid sidechain were synthesized and linked through diamide formation in order to target both sites. Diamine spacer chain lengths from C2 to C6 between both acid entities were chosen as they suggest the most promising interactions with the LBD and the CABS.
In the context of the synthesis of the OH-substituted DES-derivatives, an interesting intramolecular cyclisation reaction was identified. The boron tribromide mediated methyl ether cleavage of the DES derivative with an ethyl-hexanoate sidechain was accompanied by a Friedel-Krafts-Acylation followed by enolisation and dehydration leading to an internal alkyne. MS and 2D NMR experiments were performed to gain further information about the product and the reaction mechanism.
The main goal of developing novel ER inhibitors is the design of anti-breast cancer agents against ER-positive tumors that do not respond to currently available antihormonal therapy. A combined LBD and coactivator inhibitor may overcome this drug resistance. An additional aim of a dual mechanism of action is to achieve a more specific targeting of the ER-alpha leading to higher potency and less adverse effects.

1. Katzenellenbogen, B. S. (1996) Biology of Reproduction 54 (2): 287-293.
2. Moore, T. W., Mayne, C. G. and Katzenellenbogen, J. A. (2010) Mol Endocrinol 24 (4): 683-95.
3. Mirkin, S. and Pickar, J. H. (2015) Maturitas 80 (1): 52-57.
4. Wang, Y., et al. (2006) Proc Natl Acad Sci U S A 103 (26): 9908-11.
5. Shan, M. Dissertation (2011) Freie Universität Berlin

P-5

Predicting hyperbilirubinemia by combining extended connectivity fingerprints (ECFPs) and liver transporter inhibition profiles

Eleni Kotsampasakou[a], Sylvia Escher[b] and Gerhard F. Ecker[a]

[a]*University of Vienna, Department of Pharmaceutical Chemistry, Althanstrasse 14, 1090 Vienna, Austria*
[b]*Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Nikolai-Fuchs-Strasse 1, 30625 Hannover, Germany*

Hyperbilirubinemia is the pathological condition of accumulation of bilirubin in sinusoidal blood, which is a natural product of heme catabolism. It can be an indication of drug induced liver injury and it is dangerous because it may cause neural and non-neural organ dysfunction. Under normal conditions, bilirubin is taken up by OATP1B1 and OATP1B3, two transporters residing on the basolateral membrane of the hepatocyte. Thus, a potential inhibition of those transporters would lead to the increase of bilirubin in the blood and eventually cause hyperbilirubinemia. [1]
The aim of our study was to generate classification models for hyperbilirubinemia and to evaluate if the addition of predicted transporter inhibition profiles as descriptors increase the performance of potential models. Two datasets were exploited, one comprising 214 compounds (55 positives and 159 negatives for hyperbilirubinemia) containing data for animals (mostly rodents) provided through the eTOX project, and one on a human dataset of 835 compounds (containing 86 positives and 749 negatives) from public literature. [2]
For modeling the dataset several base classifiers, such as SMO, Random Forest, Naïve Bayes, kNN (k=5), J48 tree and Logistic regression, were used, always in combination to the MetaCost meta-classifier, due to the imbalance of the datasets. Meta-classifiers usually enhancing the performance of base-classifiers, such as Stacking and Boosting, were also explored. As descriptors for modeling all 2D MOE descriptors, the volsurf 3D descriptors and ECFP6 and ECFP4 fingerprints (with and without attribute selection) were considered.
For the case of animal data, generally merely moderate results were obtained. Using 10-fold cross validation, the best performance was obtained using Logistic regression with MetaCost and ECFP6 fingerprints (accuracy = 0.561 and AUC= 0.584), while the inclusion of OATP inhibition as additional descriptors does not substantially differentiate the performance (accuracy = 0.535 and AUC= 0.602).
For the human data, considerable better models could be obtained. The best performing classifier for 10-fold cross validation was SMO (RBF kernel) with MetaCost, using ECFP6 fingerprints (accuracy = 0.679 and AUC= 0.685), while the inclusion of OATP inhibition as additional descriptors does not substantially differentiate the performance (accuracy = 0.674 and AUC= 0.687).
In general it is difficult to extrapolate animal toxicity effects from human transporter models, as well as from *in vivo* to *in vitro* data. An additional factor of difficulty is that behind toxicity endpoints, such as hyperbilirubinemia, there are multiple mechanisms underlying and also the technical issues with assays measuring bilirubin in blood. However, careful data curation can give moderate results for predicting hyperbilirubinemia with animal data, while there is improvement with human data.

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115002 (eTOX), as well as from the Austrian Science Fund, grant F3502.

1. Chang et al., 2013, Mol Pharmaceut, 3067-3075
2. Liu, Zhichao et al., PLoS Comput Biol, 7.12 (2011): e1002310

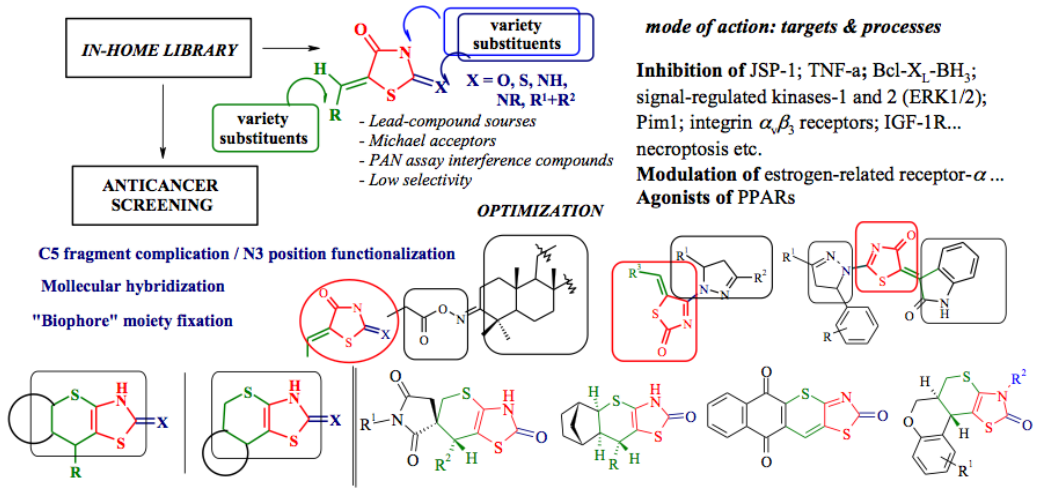
P-6

5-Ylidene-4-thiazolidinones as new anticancer agents: pro et contra

Roman Lesyk, Borys Zimenkovsky, Danylo Kaminskyy, Anna Kryshchyshyn, Dmytro Havrylyuk, Nataliya Zelisko, Oleg Devinyak, Dmytro Khyluk, Olexandra Roman, Andrii Lozynskyi, Maryan Lelukh, Magdalena N. Wojtyra

Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv, Ukraine

4-Thiazolidinone core is the known privileged scaffold in medicinal chemistry and can be treated as powerful tool in the design of new drug-like molecules, especially within structure-based design. Among diversity of 4-thiazolidinone derivatives 5-ylidene-4-thiazolidinone-based compounds are of special interest (majority of 4-thiazolidinone based drugs, drug-candidates and lead compounds belong to the mentioned sub-types). It's reflected in the thesis about crucial role of the C5 substituent nature in the biological activity realization. While ylidene fragment conjugation to the C4 carbonyl group makes compounds to be Michael acceptor. This feature characterizes molecules as frequent hitters or pan assay interference compounds that may be useless in drug discovery process because of possible insufficient selectivity. While, such Michael acceptors are among the most effective activators of Nrf2, which opens new perspectives in the treatment of inflammation, cancer, etc. Thus, the aim of the project is utilization of 5-ylidene-4-thiazolidinone scaffold in the search for new anticancer agents.



Following of the chemical diversity of 4-thiazolidinones the in-house library of new heterocycles have been designed and synthesized (more 5000 compounds). Anticancer activity screening within USNCI DTP protocol led to SAR database formation; lead-compounds identification; design of focused sub-libraries; formation and validation of hypotheses for structure optimization: *i*) complications of C5 fragment and/or functionalization of N3 position; *ii*) creation of the hybrid molecules; *iii*) fixation of 5-ylidene-4-thiazolidinones in fused heterocycles via annulation (thiopyrano[2,3-*d*]thiazoles were found as a cyclic isosteric mimetics of 5-arylidene-4-thiazolidinones); *iv*) the leukemia panel was detected to be the most sensitive among all cancer cell lines. Following the *in silico* and pharmacological data for the investigation of molecular mechanism of anticancer effect the argument in favor of the apoptotic related and mild prooxidant actions for active compounds have been found.

P-7

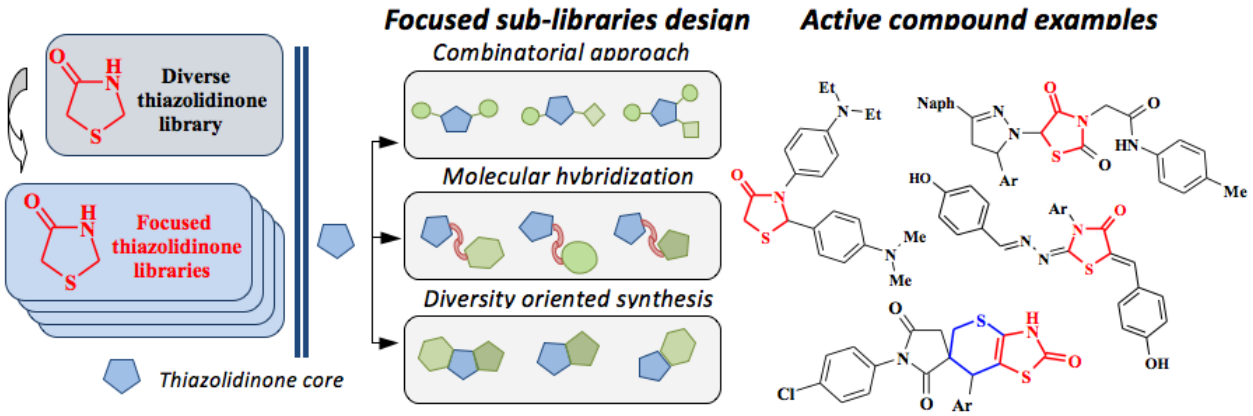
Design of the new antitrypanosomal agents bearing 4-thiazolidinone core

Danylo Kaminskyy[a], Anna Kryshchyshyn[a], Dmytro Havrylyuk[a], Natalya Zelisko[a], Maryan Lelukh[a], Phillippe Grellier[b], Roman Lesyk[a]

[a]Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv, Ukraine; [b]National Museum of Natural History, CP 52, 57 Rue Cuvier, 75005 Paris, France.

Human African Trypanosomiasis (sleeping sickness) is among the most serious regional neglected tropical diseases caused by Trypanosoma brucei (*T.b.*) which have now spread to other continents. Despite the increasing interest in the search for new antitrypanosomals, and the impressive advances in understanding the biology of *T.b.* the treatment of trypanosomiasis urgently requires new effective and non-toxic drugs (since eflornithine was approved in the 90^s no new drugs were marketed). Following the current trends in the new antitrypanosomanl agents design thiazolidinones are of special interest as *i*) mimics of thioureas/ thiosemicarbazones *ii*) attractive scaffolds for structure-based design. Limitations of highly active ligands (to validated targets) application are due to low effectiveness in vivo or toxicity. The present project is an extension of our ongoing efforts [1-4] towards search new antiparasitic agents.

The project realization involves the several stages: *1*) primary screening of antitrypanosomal activity (*T. brucei*) of the diversity thiazolidinones library; *2*) SAR analysis, design and synthesis of focused sub-libraries within combinatorial and privileged-substructure-based diversity oriented synthesis strategies and molecular hybridization; *3*) sub-libraries screening, hits and leads identification; *4*) (Q)SAR(P) analysis and formation of the direction for structure optimization; 5) *in depths* study of mode of action, toxicity evaluation. The set of thiazolidinone-based compounds with anticancer activity was involved into the study following the new findings about the simultaneous anticancer and antitrypanosomal activities.



Based on preliminary results the focused sub-libraries were selected, set of hit- and lead-compounds were detected (the most efficient trypanocidals (*in vitro*) were found to be active against ***Trypanosoma brucei brucei*** **and** ***Trypanosoma brucei gambiense*** with IC_{50} 0.01–0.10 μg/mL) as well as some modifications items were discovered.

1. Havrylyuk D, Zimenkovsky B, Karpenko O, Grellier P, Lesyk R (2014) Eur. J Med. Chem. 85: 245 – 254.
2. Kryshchyshyn A, Kaminskyy D, Grellier P, Lesyk R (2014) Eur. J. Med. Chem. 85: 51 – 64.
3. Zelisko N, Atamanyuk D, Vasylenko O, Grellier P, Lesyk R (2012) Bioorg. Med. Chem. Lett. 22: 7071 – 7074.

P-8

Generation and evaluation of DARPin-siRNA conjugates for receptor-specific tumor targeting

Cornelia Lorenzer, Sonja Streußnig, Emilia Tot, Ernst Urban, Johannes Winkler

Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Therapeutic oligonucleotides, particularly antisense oligonucleotides and small interfering RNA (siRNA), are promising agents for the treatment of a number of diseases such as cancer, viral infections, and autoimmune diseases, since they induce highly specific downregulation of gene expression. However, the use of oligonucleotides for medicinal applications still constitutes a barrier, as they suffer from poor pharmacokinetic properties and insufficient cellular uptake in diseased tissues. In recent years, pharmacokinetic properties and cellular uptake could be stepwise improved by chemical modifications of the lead structure or by development of advanced delivery systems equipped with a ligand for targeting tissues. Selective and high-affinity receptor binding as well as easy production, purification and chemical derivatization are important requirements for targeting ligands. DARPins are high-affinity binding proteins with advantageous biophysical properties including remarkable chemical and thermal stability, low aggregation density and high-yield expression in the cytoplasm of Escherichia coli [1]. A promising approach for targeted oligonucleotide delivery are bioconjugates, which enable traditional pharmaceutical quality assessment [2].

Here we present the generation and *in vitro* evaluation of DARPin-based oligonucleotide conjugates. For this purpose, the sense strand of siRNA was attached to a cysteine- or azide-modified DARPin using different conjugation chemistries. The modular structure of the DARPins enables site specific attachment to unique cysteins or artificial azide-modified amino acids. Conjugation procedures were carefully monitored and optimized for high yields. A novel, two-step affinity purification method was developed for isolating bioconjugates from the reaction mixtures. First, Ni-NTA affinity chromatography was used for His-tag binding and separation from unreacted oligonucleotides. Subsequently, unreacted protein was removed through binding of the bioconjugate to an overlapping complementary oligonucleotide strand. Gel electrophoresis and size-exclusion chromatography showed successful purification through this two-step affinity protocol. The siRNA counter strand easily hybridized to the conjugates by simple addition in native buffers, and allows for facile fluorescent tagging of the oligonucleotide cargo for evaluation of receptor binding and cellular uptake. Using cell-based assays with receptor-positive and –negative cell lines, we showed EpCAM-specific binding of all intact DARPin-siRNA conjugates by flow cytometry.

In conclusion, we successfully developed the methodology for generation of DARPin siRNA conjugates. The efficient and selective binding make DARPin-siRNA conjugates promising systems for targeted delivery.

1. Binz HK, Amstutz P, Kohl A, Stumpp MT, Briand C, Forrer P, et al. (2004) Nat. Biotechnol. High-affinity binders selected from designed ankyrin repeat protein libraries 22(5):575-82.
2. Winkler J. (2013) Ther deliv. Oligonucleotide conjugates for therapeutic applications 4(7):791-809.

P-9

Antitumour active cobalt alkyne complexes derived from acetylic salicylic acid: studies on impact of fluorination and chlorination of Co-ASS

Victoria Obermoser, Carina Schuster, Sarah-Maria Lettenbichler, Elisabeth Kircher, Valentin Braun, Ronald Gust

CCB – Centrum für Chemie und Biomedizin, Institute of Pharmacy, Department of Pharmaceutical Chemistry, University of Innsbruck, Innrain 80-82, A-6020 Innsbruck, Austria

Background. [(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS), a derivative of the irreversible COX-1/-2 inhibitor acetylic salicylic acid (ASS) chelated to cobalt, demonstrated high growth-inhibitory potential against various tumour cells with interference in the arachidonic acid cascade as probable mode of action. [1] We modified the ASS moiety esterified by a propargyl group and obtained its chlorinated and fluorinated derivatives, respectively. In a comprehensive structure activity relationship study we investigated the new complexes.
Aim. The aim of our study is to identify the impact of a fluorine or chlorine substitute in different positions of the acetylic salicylic acid moiety on cytotoxic activity, COX inhibition in general and COX isoenzyme selectivity in particular compared to the lead structure Co-ASS.
Methods. Compounds were evaluated for cytotoxicity in breast [MCF-7 (hormone dependent), MDA-MB-231 (hormone independent)] and colon cancer [HT-29] cell lines and for COX-1/-2 inhibitory effects at human recombinant or ovine isoenzymes. For selected compounds with strong COX-1/-2 inhibition, the major COX metabolite prostaglandine E2 (PGE2) was quantified in arachidonic acid-stimulated MDA-MB 231 breast tumor cells via enzyme immunoassay (EIA).
Results. Whereas the ligands only showed an insignificant cytotoxic effect, cobalt complexes were able to inhibit tumor cell growth in all cell lines to a larger extent. Further, complexation of ligands to cobalt led to a tenfold increase in the potency to inhibit COX-1/-2 compared to ligand only. By fluorinating and chlorinating the ASS propargylester moiety, we could achieve a shift in COX selectivity and a cell line specific cytotoxicity comparable to the one of Co-ASS. In general, the position of the halide contributed strongly to these cytotoxic and COX-inhibitory effects. The chlorinated derivatives, however, showed different cyclooxygenase inhibition and cytotoxicity patterns than the fluorinated derivatives. Compounds with a distinct inhibition of COX but low cytotoxic potential in the cell based assay, indicate that the cell membrane might be the limiting factor for the action of some of the new CoASS derivatives.
Discussion. Halogenated Co-ASS derivatives are a new approach in the development of new antineoplastic agents with favored inhibition of COX-2, the isoenzyme with greater impact on tumor development compared to COX-1. Due to the low cytotoxicity of cobalt complexes in human in general, also the newly synthesized compounds might be well-tolerated and are, therefore, promising.

1. Ott I, Kircher B, Bagowski CP, Viecken DH, Ott EB, Will J, Bensdorf K, Sheldrick WS, Gust R (2009) Angew Chem 48: 1160-1163.

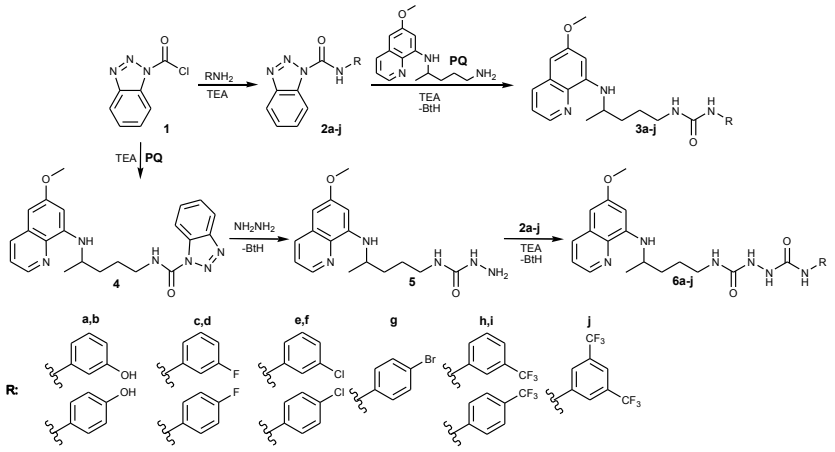
P-10

Synthesis and characterization of novel primaquine ureas and semicarbazides

Ivana Perković, Ana Palajsa, Petra Gilja, Kristina Pavić, Branka Zorc

Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10 000 Zagreb, Croatia

Primaquine (PQ), a member of the 8-aminoquinoline group, is a well known antimalarial drug with pronounced antitumour activity as well [1]. Recently, we have reported several papers describing the synthesis, antitumour and antioxidant activity of various primaquine derivatives. The majority of our previously synthesized primaquine semicarbazides showed either prominent cytostatic activity towards all the tested cell lines or high selectivity towards MCF-7 cells with IC_{50} values in the low micromolar range. Urea derivatives were generally less active than their semicarbazide analogues, but still selective towards MCF-7 cells [2-4]. The objective of these studies was to optimize antitumour activity of primaquine derivatives. Among the synthesized compounds, primaquine semicarbazides and ureas with halogenated phenyl moieties showed significant cytostatic activity [4]. Based on these findings, we found it worth to prepare urea and semicarbazide primaquine derivatives bearing various phenyl moieties with electronegative substituents (halogens or oxygen) at the one and primaquine moiety at the other end.



Scheme outlines the general preparative route. The starting compound benzotriazole carboxylic acid chloride (1) was used for the preparation of both active ureas (2a-j) and benzotriazolide (4). compounds 3a-j and 6a-j were obtained in the reaction of primaquine or primaquine semicarbazide (5) and corresponding active ureas (2a-j). Compound 4 with hydrazine hydrate gave primaquine semicarbazide (5). Structures of newly prepared primaquine derivatives were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy and MS. Evaluation of their biological activity is in progress.

References:
1. Duffy R, Wade C, Chang R (2012) Drug Discov. Today 17: 942 – 953.
2. Šimunović M, Perković I, Zorc B, Ester K, Kralj M, Hadjipavlou-Litina D, Pontiki E (2009) Bioorg. Med. Chem. 17: 5605 – 5613.
3. Perković I, Tršinar S, Žanetić J, Kralj M, Martin-Kleiner I, Balzarini J, Hadjipavlou-Litina D, Katsori AM, Zorc B (2013) J. Enzyme Inhib. Med. Chem. 28: 601 – 610.
4. Pavić K, Perković I, Cindrić M, Pranjić M, Martin-Kleiner I, Kralj M, Schols D, Hadjipavlou-Litina D, Katsori AM, Zorc B (2014) Eur. J. Med. Chem. 86: 502 – 514.

P-11

Conformational Sampling with iCon: Algorithm and Performance Assessment

Thomas Seidel[a], Giulio Poli[b], Thierry Langer[a]

[a]*Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria;* [b]*Department of Pharmacy, University of Pisa, 56126 Pisa, Italy*

We will describe the algorithm and performance assessment of our newly developed conformer generator iCon. iCon was implemented for the pharmacophore modelling software LigandScout [1] and uses a systematic, knowledge-based approach for the generation of small molecule conformer ensembles.

We employed different metrics and used two data sets of high-quality X-ray structures from the Protein Data Bank (PDB) [2] and Cambridge Structural Database (CSD) [3] to validate iCon's performance in reproducing experimental crystallographic conformations in comparison with OpenEye's conformer generator OMEGA [4]. Several setting patterns were tested in order to identify the most suitable ones for iCon; equivalent settings were tested for OMEGA to compare the results.

iCon showed its best performance in the reproduction of experimental poses of CSD compounds through small conformational ensembles while OMEGA proved to be more effective in the sampling of the PDB data set, comprising ligands endowed with higher conformational flexibility. OMEGA showed to be slightly faster than iCon. Moreover, for equivalent settings, iCon always achieved similar results in terms of performance through a smaller number of generated conformers, thus showing the potential of speeding up any virtual screening or pharmacophore modeling process based on its conformational analysis. Overall, the study proved that iCon is a powerful new conformer generator that is able to generate reasonable low-energy conformational ensembles of diverse organic compounds with low computational demand.

References:
1. LigandScout, version 4.0, Inte:Ligand GmbH, Vienna, Austria, www.inteligand.com, 2015.
2. Berman H.M., Westbrook J., Feng Z., Gilliland G., Bath T. N., Weissig H., Shindyalov I. N., Bourne P. E. (2000) Nucleic Acids Res., The protein Data Bank 28: 235-242.
3. Allen F. H. (2002) Crystallogr. Acta, Sect. B: Struct. Sci., The Cambridge Structural Database: a Quarter of a Million Crystal Structures and Rising 58: 380-388.
4. MEGA, version 2.4.6. OpenEye Scientific Software Inc., Santa Fe, NM, USA, www.eyesopen.com, 2013.

P-12

Linear polyethylenimine based nanocarriers for gene therapy: single nanoparticle tracking analysis, production upscale and microspraying

Alexander Taschauer[a], Antonia Geyer[a], Julia Maier[a], Sebastian Gehrig[a], Jakob Sperhansl[a], Karoline Völckers[a], Haider Sami[a], Manfred Ogris[a]

[a]*Laboratory of MacroMolecular Cancer Therapeutics (MMCT), Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*

Nucleic acid delivery for gene therapy applications is challenging because of the various biological barriers offered against nucleic acids. Non-viral delivery vehicles are safer and offer numerous advantages in comparison to viral gene delivery approaches. Linear polyethylenimine (LPEI) is a 'gold standard' for synthetic gene delivery systems because of its high transfection efficiency, which is achieved by complexing nucleic acids into nanometer-sized particles, also termed as polyplexes. In the present work, LPEI polyplexes based on luciferase reporter genes were studied for a) their biophysical properties by single nanoparticle tracking analysis (NTA), b) upscaling the production by syringe pump mediated synthesis protocol and c) in vivo gene delivery by intravenous injection or microspray enabled aerosolization. A powerful strategy to determine the transfection activity and transgene expression level in vitro and in vivo is the use of luciferase-based reporter genes, such as *Gaussia* luciferase and firefly luciferase, which can be detected through the reaction with their respective substrates. Due to the dependency of the properties of polyplexes on their surrounding environment and the influence of the particle size on the transfection efficiency, detailed investigation of their biophysical properties is crucial for their quality control. NTA is a relatively recent technique which tracks single nanoparticles, thereby giving more detail on the nanoparticle population characteristics for size and zeta potential when compared to the routinely used dynamic light scattering system. The use of a syringe pump for synthesizing polyplexes ensured an upscaled synthesis which is characterized by a controlled and reproducible mixing resulting in nanoparticles with a narrow size distribution. Besides the successful reporter gene delivery in vivo by intravenous route, we investigated the possibility to use LPEI based polyplexes as an aerosol for potential lung delivery applications. For aerosolization of polyplexes, we utilized a microsprayer which is designed for fast and targeted intratracheal administration to mouse. Aerosolized polyplexes were studied for their biophysical properties by NTA and in vitro gene transfer efficiency. Taken together, linear polyethylenimine is a versatile and powerful transfection agent in vitro and in vivo for both systemic and intratracheal administration.

P-13

Quantification of the relative percentage distribution of THCA and Δ9-THC in herbal cannabis seized by Austrian police: Stability tests at different storage temperatures

Magdalena Taschwer, Martin G. Schmid

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz, Universitätsplatz 1, A-8010 Graz, Austria

Cannabis is doubtless one of the controversially discussed and misused drugs since its legalization in some of the US. According to the European Drug Report 2015, cannabis is the most commonly seized drug in Europe which is emphasized by the fact that 80% of the seizures are herbal cannabis or resin [1]. In many contributions it is confirmed as a gateway drug [2, 3], which is increasingly misused regardless of age and social classes.
The flowering part of the female cannabis plants contain the highest concentration of cannabinoids. The major cannabinoid is Δ^9-tetrahydrocannabinol (Δ^9-THC), which is mainly responsible for the psychoactive effects. Tetrahydrocannabinolic acid (THCA), its inactive biosynthetic precursor, is present in different quantities in fresh plant material. Due to influence of high temperature, for example during smoking, cooking or baking it decomposes to Δ^9-THC (Fig.1).

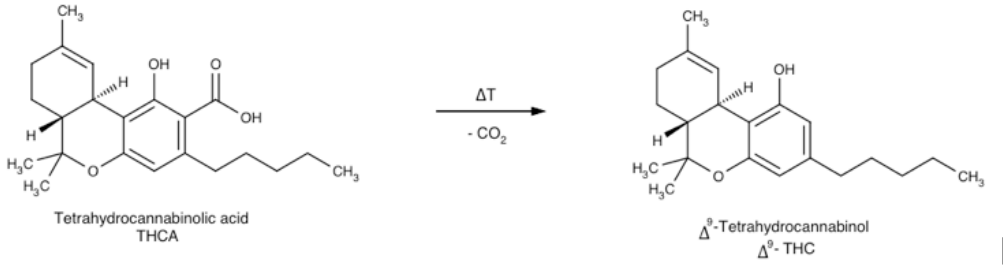


Fig.1 Biosynthetic decomposition of THCA to Δ^9-THC through heating (ΔT).

In the present research, a determination of the relative percentage distribution of the two cannabinoids Δ^9-THC and THCA in 29 herbal cannabis samples seized by Austrian police was performed. Moreover, the impact of storage temperature on the decomposition process of THCA to Δ^9-THC was carried out. Quantification was conducted by HPLC-UV using a mobile phase consisting of a 25 mM triethylammonium phosphate buffer (pH 3.0) and acetonitrile (36:64). A common LiChrospher® 100 RP-18 column served as stationary phase.
For stress tests at low as well as high temperature, herbal samples were stored in a freezer or in a drying cabinet for a specified time period.
Stability tests showed that storage only at 100 °C and 150 °C led to a complete decarboxylation of THCA within short time. Storage at ambient temperature caused slight changes of Δ^9-THC/ THCA ratio while storage for some months in the freezer did not influence the percentage distribution.

1. EMCDDA (2015) European Drug Report, Trends and developments.
2. Mayet A, Legleye S, Falissard B, Chau N (2012) Addict Behav. 37: 160-166.
3. Secades-Villa R, Garcia-Rodriguez O, Jin CJ, Wang S, Blanco C (2015) Int J Drug Policy. 26: 135-142.

P-14

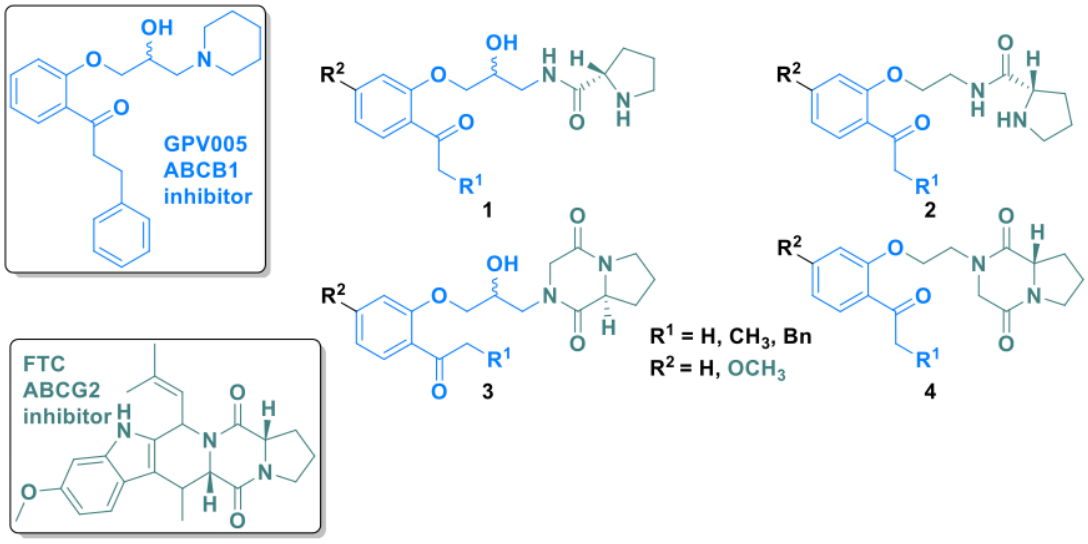
Propafenone variations: Probing selectivity between P-glycoprotein and BCRP

Theresa Weigl-Pollack[a], Anna Cseke[a], Katrin Wlcek[a], Floriane Montanari[a], Karl Kuchler[b], Ernst Urban[a] and Gerhard F. Ecker[a]

[a]Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria, [b]Department of Medical Biochemistry, Max F. Perutz Laboratories, , Medical University Vienna, Dr. Bohr-Gasse 9/2, A-1030 Vienna, Austria

Multispecific drug efflux pumps have been recognised as one major cause for resistance to anticancer treatment by effluxing cytotoxins before they are able to reach their intracellular target structures. Among others, P-glycoprotein and BCRP have been shown to be overexpressed in a large number of human tumours. Inhibition of these transport proteins has been advocated as a concept for restoration of drug sensitivity [1]. Apart from their importance in cancer, ABC transporters have also been recognized to play a vital role in tissue protection, which is shown by their expression in barrier tissues like for example the blood-brain barrier [1]. Although being both polyspecific in their ligand interaction profiles, the two transporters show distinct, but overlapping substrates and inhibitor patterns [2].

To gain further insight into the molecular basis of transporter selectivity, we picked two compounds that are selective inhibitors for P-glycoprotein (propafenone analogue GPV005) or BCRP (fumitremorgin C, FTC), respectively. By chemically morphing one scaffold to the other, we developed 4 target structures that possess structural features of both of them, but are stepwise made more similar to FTC. All compounds were tested for their inhibitory properties for both transporters, and selective inhibitors for both transporters, as well as general inhibitors were found. The results of the biological testing were compared to predictions of our in-house classification models for BCRP and Pgp inhibition and were generally in good accordance with the model.



We gratefully acknowledge financial support provided by the FWF, grant # F3502 and by the Hochschuljubiläumsstiftung der Stadt Wien, grant # H-287748/2014.

1. Dean M, Rzhetsky A, Allikmets R (2011) Genome res 11(7):1156-66.
2. Cramer J, Kopp S, Bates SE, Chiba P, Ecker GF (2007) ChemMedChem 2(12):1783-8.

P-15

Novel Zeise-type complexes with aspirin substructure as potential COX-inhibitors

Alexander Weninger, Victoria Obermoser, and Ronald Gust

CCB – Centrum für Chemie und Biomedizin, Institute of Pharmacy, Department of Pharmaceutical Chemistry, University of Innsbruck, Innrain 80-82, A-6020 Innsbruck, Austria

Background: The development of novel biologically active organometallic compounds with aspirin substructure, that also inhibit cyclooxygenase (COX) enzymes led to the synthesis of appropriate Zeise-type salts. In in vitro studies, it could be shown that Zeise's salt itself is indeed pharmacologically active and a very potent COX-inhibitor, whereas potassium tetrachloroplatinate(II) and Cisplatin caused no effect on the enzyme activity at comparable concentrations. LC-ESI-Tandem-MS proved that Zeise's salt binds to the essential amino acids Ser516 and Tyr385 in COX-1, thus impairing the function of the isoenzymes [1][2].
Aim/Object: In order to increase selectivity for COX-2 the aspirin substructure was modified resulting in novel Zeise-type complexes. These complexes were investigated for their cytotoxicity and COX-1/2 selectivity and inhibition of the COX isoenzymes. The influence of structural differences in these anionic platinum complexes on cytotoxicity and COX selectivity/inhibition were examined.
Methods: Novel Zeise-type complexes were characterized via nuclear magnetic resonance spectroscopy and electrospray ionization mass spectrometry. Prior to testing these substances for pharmacological properties in vitro using modern biological techniques, purity determination and stability tests in water were done via HPLC. Due to the strong trans-effect of the alkene a substitution of a chlorido ligand by water is possible. Another substitution reaction might be the exchange of the alkene in media with high Cl- concentration [3].
Results: A set of complexes were selected as reference and compared to the new complexes regarding cytotoxicity and COX-1/2 selectivity/inhibition. Structural differences of the aspirin substructure influence the stability of the Zeise-type complexes strongly, but lead to a higher COX-1/2 selectivity.
Conclusion: In conclusion, it could be shown that Zeise-type complexes are a potential class of non-toxic COX-inhibitors, whose COX selectivity and stability can be influenced by the size of the aspirin substructure in the platinum complex.

1. Meieranz S, Stefanopoulou M, Rubner G, Bensdorf K, Kubutat D, Sheldrick WS, Gust R (2015) Angew. Chem.: 127/9: 2876 – 2879
2. Ott I, Gust R (2007) Arch. Pharm. 340: 117 – 126
3. Joy JR, Orchin M (1960) Z. Anorg. Allg. Chem. 305: 236 – 240

P-16

Testing the robustness of pharmacophore models using molecular dynamic simulations

Marcus Wieder[a,b] , Thomas Seidl[a] , Stefan Boresch[b] and Thierry Langer[a]

[a] *Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna*
[b]*Department of Computational Biological Chemistry, University of Vienna, Waehringer Str. 17, A-1090 Vienna*

Structure-based pharmacophore models of a ligand in contact with a protein have proven to be highly useful for in-silico drug discovery and lead optimization [1]. However, the static picture of the protein-ligand structure only shows a limited view of reality. Molecular systems are inherently dynamic and display a wide range of motion. Small molecules can exhibit bond rotation, stretching and bending and protein motion spans from simple side chain rotation to large backbone rearrangements that can even alter the secondary structure of the protein. Molecular dynamic simulations provide in great detail and sufficient accuracy the motion of individual atoms as a function of time, thus providing a great tool for examining the actual dynamic of the system. [2, 3] Combining structure-based pharmacophore models with molecular dynamic simulations of protein-ligand complexes can help determining the robustness of the pharmacophore model obtained from the crystal structure.

The molecular dynamics software CHARMM (Chemistry at HARvard Macromolecular Mechanics) and CHARMM-GUI was used to obtain 20ns molecular dynamic simulations of 6 protein-ligand complexes (PDB-Code: 1J4H, 1XL2, 2HZI, DRD3, 1UYG, 3LAN) [4, 5]. LigandScout was used to prepare pharmacophore models for every 5ps of the simulation (resulting in 2,000 pharmacophore models for one simulation) [6]. The python package 'pharmacophore-analysis' (available on https://github.com/Guillopflaume/phanalysis), which was developed by the author for the purpose of combining pharmacophore models with molecular dynamic simulations, was applied for frequency analyses of pharmacophore features. This frequency analysis was used to weight the importance of pharmacophore features and change the initial pharmacophore model in order to better represent these weights.

In the poster we will present the results of this analysis. In general, the changes in the pharmacophore model are very dependent on the system that was studied and on the overall dynamics. We were able to refine static pharmacophores for selected systems to obtain better screening results. Also the ability of the pharmacophore model to distinguish between known actives and decoys for the system was improved for some of the studied systems.

1. Sanders, Marijn P. a., McGuire, Ross, Roumen, Luc, de Esch, Iwan J. P., de Vlieg, Jacob, Klomp, Jan P. G., & de Graaf, Chris. 2012. From the protein's perspective: the benefits and challenges of protein structure-based pharmacophore modeling. MedChemComm, 3(1), 28.
2. Karplus, Martin, & McCammon, J Andrew. 2002. Molecular dynamics simulations of biomolecules. Nature structural biology, 9(9), 646–52.
3. Feixas, Ferran, Lindert, Steffen, Sinko, William, & McCammon, J. Andrew. 2014. Exploring the role of receptor flexibility in structure-based drug discovery. Biophysical Chemistry, 186, 31–45.
4. Jo, S, Kim, T, Iyer, VG, & Im, W. 2008. CHARMMGUI: a webbased graphical user interface for CHARMM. Journal of computational chemistry.
5. Brooks, BR, & Brooks, CL. 2009. CHARMM: the biomolecular simulation program. Journal of computational chemistry, 30(10), 1545–1614.
6. Wolber, Gerhard, & Langer, Thierry. 2005. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. Journal of chemical information and modeling, 45(1), 160–9.

P-17

Synthesis of amino acid variations for the generation of short α-melanocyte stimulating hormone analogues

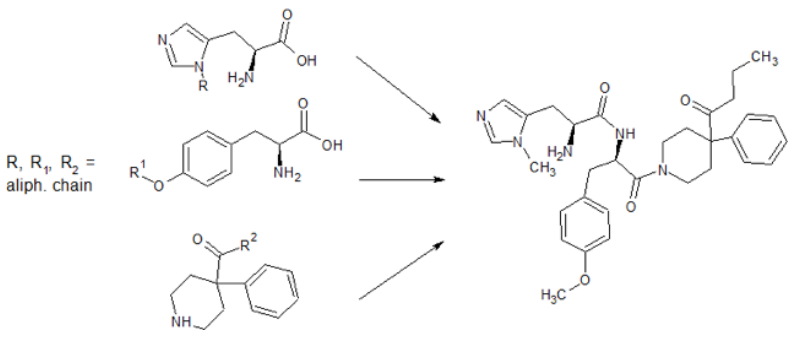
Elisabeth Wurzer-Priester, Philipp Wagner, Sebastian Schober, Martin Kratzel

Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Aim of the work: The skin is the largest organ of the human body and provides, amongst other functions, protection against UV-irradiation. The natural mechanism of skin pigmentation is activated after UVR exposure and follows a paracrine/autocrine pathway. The first important part is α-melanocyte stimulating hormone (α-MSH) activating the human melanocortin 1 receptor (hMC1R) and thus inducing the expression of enzymes for melanin production. Without the presence of α-MSH, melanocytes are not able to respond to UVR and therefore fail to produce the photoprotective melanin [1,2]. α-MSH itself is not available as a drug because of its instability against proteases, therefore simplified analogues, possessing a good stability, low molecular weight and high affinity to hMC1R are wanted [3].

Applied methods: Based on a template found in the literature, a small peptide consisting of three amino acids is the starting point of the investigation. Via classic organic synthesis variations of the amino acids were generated in order to prepare a substance library of varying small peptides.

Main results: We have synthesized a series of amino acid analogues with varying substituents on key positions such as the ring nitrogens in the histidine derivates. Tyrosine compounds have been alkylated at the alcohol-oxygen and the piperidine moiety was generated using different aliphatic chain lengths for the keto group.



Conclusion: We have managed to build a substantial stock of amino acid building blocks which are going to be combined with each other via solid-phase peptide synthesis in order to obtain a library of tripeptides. These substances are then subjected to biological testing on a melanoma cell line for their activity at the hMC1R.

[1] Abdel-Malek, Z. A., A. L. Kadekaro, R. J. Kavanagh, A. Todorovic, L. N. Koikov, J. C. McNulty, P. J. Jackson, G. L. Millhauser, S. Schwemberger, G. Babcock, C. Haskell-Luevano and J. J. Knittel (2006). FASEB J 20(9): 1561-1563.
[2] Nasti TH, Timares L. Photochem Photobiol. 2015;91(1):188-200.
[3] Hruby VJ, Cai M, Cain J, Nyberg J, Trivedi D. Eur J Pharmacol. 2011;660(1):88-93.

Selective inhibition of proinflammatory action of oxidative stress via non-antioxidant mechanisms

Valery Bochkov[a,b], Markus Binder[b], Murthy Bulusu[b]

[a]*Institute of Pharmaceutical Sciences, University of Graz, Humboldtstrasse 46/III, Graz 8010, Austria;* [b]*Center for Physiology and Pharmacology, Schwarzspanierstrasse 17, Vienna 1090, Austria*

Oxidized phospholipids (OxPLs) represent an emerging class of lipid mediators generated by enzymatic or non-enzymatic oxidation of esterified polyunsaturated fatty acids. Multiple studies have documented proinflammatory action of OxPLs in a number of in vitro and in vivo models. However, the intracellular signaling mechanisms mediating these effects are only partially understood and no specific inhibitors of toxic and proinflammatory action of OxPLs are currently known. We identified a chemical scaffold (named MB) that selectively inhibited production of proinflammatory IL-8 by OxPLs but had minimal effects on the action of recognized inflammatory agonists such as cytokines (TNF, IL-1) or bacterial products (lipopolysaccharide). In addition, MB compounds inhibited induction of IL-8 by other lipid oxidation products and non-lipid oxidants, including hydroxynonenal, electrophilic prostaglandins, hydrogen peroxide and cigarette smoke extract. The data suggest that MB compounds may target a common signaling step mediating proinflammatory action of oxidative stress but not that of cytokines or bacterial products. Potentially, this finding opens a possibility for targeted antagonism of proinflammatory action of lipid oxidation products without impairing general innate immune response. Analysis of several currently known mechanisms of proinflammatory action of OxPLs showed that none of them is likely to be targeted by MB-7. The study suggests new approaches for pharmacological protection against oxidative stress using non-antioxidant compounds.

P-19

Using flow cytometry for evaluation of structure-activity relationships of ABCB1 and ABCG2 inhibitors

Anna Cseke[a], Katrin Wlcek[a], Theresa Weigl-Pollack[a], Floriane Montanari[a], Lene Visvader[a], Ernst Urban[a], Karl Kuchler[b], Peter Chiba[c] ,Gerhard F. Ecker[a]

[a]Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [b]Department of Medical Biochemistry, Max F. Perutz Laboratories, Medical University Vienna, Dr. Bohr-gasse 9/2, A-1030 Vienna, Austria; [c]Medical University Vienna, Institute of Medical Chemistry, Währingerstrasse 10, A-1090 Vienna, Austria

ABCB1 and ABCG2 are transmembrane transporter proteins belonging to the ABC (ATP binding cassette) transporter superfamily. As ATP dependent efflux pumps, they mediate the transport of numerous compounds, including drugs in clinical use and metabolites, out of cells. Based on their physiological expression in tissues with barrier functions (GI tract, liver, kidney, and blood-brain barrier), both transporters are involved in drug absorption, distribution and excretion and therefore are important determinants in drug action and drug-drug interactions. Additionally, these ABC proteins are associated with cancer multidrug resistance due to their ability to transport anticancer agents out of tumor cells.

Although the overlapping substrate specificity and the selective inhibition of ABCB1 and ABCG2 have been extensively studied, the knowledge about physico-chemical properties responsible for inhibition activity is still scarce. Therefore, we investigated the effect of derivatives of propafenone, which is a selective ABCB1 inhibitor, on ABCB1 and ABCG2 function in order to identify chemical features influencing inhibition potency. Performing fluorescent substrate accumulation assays using ABC overexpressing cell lines and flow cytometry, IC50 values were evaluated and compared to gain a better insight into the relation between the chemical structure and the inhibition profile of propafenone derivatives.

A comparative effect of nicotine and UV radiation on melanization process in HEMn-DP and HEMn-LP melanocytes

Marcin Delijewski, Dorota Wrześniok, Artur Beberok, Jakub Rok, Michał Otręba, Ewa Buszman

Department of Pharmaceutical Chemistry, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland

Background: Nicotine is a commonly known alkaloid being a main agent in smoking cessation therapies and promising substance in pharmacological attempts because of its presumed neuroprotective properties [1]. Nicotine forms complexes with melanin what may lead to its accumulation in human pigmented tissues [2-3]. Melanin biopolymer is produced in melanocytes in a multi-step process called melanogenesis, in which the key enzyme is tyrosinase [4]. The role of melanin is to protect cells from UV radiation by absorbing energy as well as to act as an antioxidant agent and free radicals scavenger [5].
Aim: The aim of this study was to examine the simultaneous effect of nicotine and UV radiation on melanization process in cultured normal human melanocytes dark and light pigmented.
Methods: The normal human epidermal melanocytes (HEMn-DP and HEMn-LP, Cascade Biologics) were exposed to nicotine in concentrations of 0.01, 0.05, 0.1, 0.5 or 1.0 mM for 24 h and UVA radiation for 15 or 30 minutes. The melanin content and activity of the main melanogenic enzyme, tyrosinase were measured spectrophotometrically in cell lysates.
Results: In dark pigmented melanocytes treated with nicotine in concentrations 0.01 mM and 0.05 mM for 24 h and exposed to UVA radiation for 30 min, melanin content increased by 32.1 and 19.6%, respectively, while after exposure to UVA radiation for 15 min, by 26.6 and 16.5%, respectively, when compared with the controls. Tyrosinase activity for these concentrations of nicotine and exposure to UVA radiation for 30 min increased by 34.5% and 17.3%, respectively, while the effect of UVA radiation for 15 minutes resulted in increase in tyrosinase activity by 28.2% and 17.7%, respectively.
After incubation of light pigmented melanocytes with nicotine in concentrations 0.5 mM and 1.0 mM for 24 h and exposure to UVA radiation for 15 min, melanin content was decreased by 8.8% and 20.0%, respectively, when compared with the controls. After exposure to UVA radiation for 30 min, only nicotine in the highest tested concentration caused decrease in melanin content by 11.1%. Tyrosinase activity for nicotine concentration 1.0 mM and exposure to UVA radiation for 15 min or 30 min decreased by 18.6% or 11.8%, respectively, when compared with the controls.
Conclusion: The obtained results may explain a potential ability of nicotine to modulate the melanization process in melanocytes *in vivo,* especially during long term exposition to nicotine, like use of nicotine replacement therapy (NRT), and UV radiation. This may be particularly significant for dark pigmented skin.

1. Williams E, Linert W (2004) In Vivo 18:391-399
2. Yerger VB, Malone RE (2006) Nicotine Tob Res 8:487-498
3. Delijewski M, Buszman E, Wrześniok D (2013) Ann Acad Med Siles 67:361-366
4. Sulaimon SS, Kitchell BE (2003) Vet Dermatol 14:57-65
5. Brenner M, Hearing VJ (2008) Photochem Photobiol 84:539-549

P-21

3D Imageable spontaneously metastasizing orthotopic cancer models and a new paradigm in anti cancer drug development

Sebastian Gehrig[a], Julia Maier[a], Simone Mezgolits[a], Antonia Geyer[a], Martina Anton[b], Eva Hellmén[c], Manfred Ogris[a]

[a]*Laboratory of MacroMolecular Cancer Therapeutics (MMCT), Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*
[b]*Institute of Molecular Immunology & Experimental Oncology, Ismaninger Str. 22 81675 München Germany*
[c]*SLU, Department of Anatomy, Physiology and Biochemistry, Box 7011, SE-75007 Uppsala, Sweden*

Despite the availability of pre clinically highly effective anti cancer therapeutics, clinical survival rate of metastasized cancer remains poor. Cancerogenesis requires the interplay of countless genetic, immunologic, environmental, toxic and random factors[1] and therapy is hampered by complex cancer physiology and anatomy[2,3] and high inter individual genetic and phenotypic variations generally denoted as tumor heterogeneity[4]. Currently wide spread used genetically engineered rodent models can only mirror certain aspects of cancer biology but are not able to reproduce the complex interplay of the mentioned factors contributing to disease development and therapy resistance. Because of this there is urgent need to refine models and analysis methods and to introduce new paradigms in the drug development path. Promising approaches are employment of rodent models generated by orthotopic implantation of tumor cells, closer mirroring human cancer biology in terms of tumor micro environment and occurrence of spontaneous metastasis[5]. Methods of multimodal 3D small animal molecular imaging allows precise longitudinal analysis of tumor load, metastases pattern and absolute quantification of viable tumor cells, representing a big step forward compared to widespread used 2D signal acquisition[6]. And, as a new paradigm, including pet dog cancer patients in the drug development path promises reliable translation into human oncology, due to strong similarities between pet dogs and humans in terms of cancer biology, exposure to risk factors and response to conventional therapy[7]. Because of this here we present a 3D combined bioluminescent and computed tomography imaging study of two spontaneously metastasizing orthotopic mammary carcinoma rodent models which can be imaged and characterized by combined bioluminescent and computed tomography. 4T1 (mouse mammary carcinoma – model already described employing 2D optical imaging)[8] and CMT-U27 (canine mammary carcinoma – in house developed model) cells were lentiviraly transduced with the luciferase gene and implanted into the mammary fat pad of female Balb/C or nude mice respectively. Following surgical removal of primary tumor, metastases could be detected in lung, liver, brain, skin, axillar and popliteal lymph nodes and lower abdomen. Radiologic findings were confirmed by pathologic and histologic examination. 3D imaging studies yielded high similarities between model cancer biology to that of human and canine mammary carcinoma. Tumor load, distribution and tumor cell viability could be longitudinally non invasively monitored by multimodal 3D imaging, thus providing a good basis for following therapy studies. Results acquired in the CMT-U27 imageable orthotopic model of canine mammary carcinoma furthermore pave the way for subsequent comparative medicine studies in pet dogs.

1. Nik-Zainal S et.al. (2012) Cell. 149: 994-1007
2. Wu M, Frieboes HB, Chaplain MA, McDougall SR, Cristini V, Lowengrub JS. (2014) J. Theor Biol.
3. Moghimi SM, Hunter AC, Murray JC (2005) FASEB J. 19:311-30
4. Turtoi A, Blomme A, Castronovo V. (2015) Bull Cancer 102:17-23
5. Devaud C et.al. (2014) Mol Ther. 22:18-27
6. Kuo C, Coquoz O, Troy TL, Xu H, Rice BW. (2007) J Biomed Opt. 12: 024007
7. Paoloni M, Khanna C. (2008) Nat Rev Cancer 8:147-56
8. Tao K, Fang M, Alroy J, Sahagian GG. (2008) BMC Cancer 8:228

P-22

Synergistic anti-cancer effects of Sorafenib and Beauvericin in cervical cancer cell lines

Daniela Heilos[a,b], Thomas Mohr[b], Rosa Lemmens-Gruber[a], Walter Berger[b,]
Rita Dornetshuber-Fleiss[a,b]

[a]*Department of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*
[b]*Institute of Cancer Research, Department of Internal Medicine I, Medical University of Vienna, Comprehensive Cancer Center of the Medical University of Vienna, Borschkegasse 8A, A-1090 Vienna, Austria*

In previous studies secondary metabolites of fungi from the genus *Fusarium* were discussed as potential chemotherapeutical agents against several malignant diseases [1][2][3]. In particular, the cyclic hexadepsipeptide Beauvericin (BEA) showed promising anti-cancer effects in the human cervix cancer derived cell line KB-3-1 [4].
In this research project, we combined BEA with the clinically approved multiple tyrosine kinase inhibitor Sorafenib (Sora) (Nexavar®), *in vitro* to assess possible synergistic effects of the combination regimen.
The cytotoxic activity of BEA and Sora alone or in combination was tested in several cervical cancer cell lines (KB-3-1, Caski, HT-3, GH354, ME180, C4-I) by MTT assays performed in triplicates. Moreover, flow cytometric apoptosis assays (JC-1 staining, AnnexinV/probidium iodide (PI)), cell apoptosis stainings (Hoechst 33258/probidium iodide (HÖ/PI)), ^{3}H-thymidine incorporation, cell cycle (PI staining) and Western blot analyses were performed to determine the signaling response after drug treatment in at least two independent experiments. Finally, human umbilical vein endothelial cells (HUVECs) were used for scratch and tube formation assays.
Consistent with the synergistic cytotoxic effects of BEA in combination with Sora observed in all tested cell lines, significantly increased mitochondrial membrane depolarization (JC-1 staining) (up to 2.5 fold, $p<0.001$) and enhanced apoptosis rates in Annexin-V/PI- (up to 4.8 fold, $p<0.05$) and HÖ/PI- stained KB-3-1 cells (up to 10.4 fold, $p<0.001$) were detected compared to single treatments with Sora. Likewise, incubation of KB-3-1 cells with both agents led to a reduction of DNA-synthesis (up to 80%) and cell cycle arrest in the S-phase after 24h and cell cycle arrest in the G2/M stage after 48h. Furthermore, Western blot analyses displayed complex effects on various MAPK pathway molecules. Tube formation and scratch assays revealed reduced formation of endothelial tubes (up to 2.9 fold, $p<0.01$) as well as impaired migration (up to 1.4 fold, $p<0.01$) of endothelial cells upon addition of subtoxic concentrations of both substances compared to single treatments.
Our results clearly demonstrate that BEA and Sora exhibit synergistic effects *in vitro* on several parameters indicative for cancer cell survival, suggesting that BEA might improve the therapeutic activity of Sora in cervix cancer. To confirm the potential clinical relevance of these findings by *in vivo* studies, xenograft experiments with KB-3-1 cells in CB-17 SCID mice are planned.

1. Jow GM, Chou CJ, Chen BF, Tsai JH (2004) Cancer Lett. 216: 165–173.
2. Dornetshuber R, Heffeter P, Kamyar MR, Peterbauer T, et al. (2007) Chem. Res. Toxicol. 20: 465 –473.
3. Lin HI, Lee YJ, Chen BF,Tsai MC, et al. (2005) Cancer Lett. 230, 248–259.
4. Dornetshuber R, Heffeter P, Sulyok M, Schumacher R, et al. (2009) Mol. Nutr. Food Res. 53: 904 – 920

P-23

Inhibition of hERG Potassium and Cardiac Sodium Channels by BIMU-8

Shahid Muhammad Iqbal[a], Hanno Gerritsmann[b], Stefan Böhmdorfer[c], Rosa Lemmens-Gruber[a]

[a]Center of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [b]Research Institute of Wildlife Ecology, Department of Integrative Biology and Evolution, University of Veterinary Medicine Vienna, Savoyenstraße 1, A-1160 Vienna, Austria; [c]Division of Chemistry of Renewable Resources, University of Natural Resources and Life Sciences, Konard Lorenz-Strasse 24, A-3430 Tulln an der Donau, Austria

Human heart contains multiple ion channels which play their part in propagation of action potential to ensure organized rhythmic contraction of the heart. Activation of cardiac sodium channels results in large inward sodium current causing depolarization while delayed rectifier hERG potassium channels impart their role in controlling the repolarization during action potential. Both of the aforementioned channels are blocked by a number of drugs which may generate arrhythmias as an adverse effect or can be used as antiarrhythmic agents for the treatment of specific arrhythmias [1]. BIMU-8 is a 5-HT4 receptor agonist which is used experimentally to counteract opioid induced respiratory depression [2]. Upon intravenous administration in rabbits (2 mg/kg), serious disturbances in ECG were recorded, which encouraged us to check its effect on cardiac ion channels.

HEK-293 cells stably expressing cardiac sodium channel $Na_v1.5$ or hERG potassium channels were maintained in MEM medium supplemented with FBS (10%), penicillin/streptomycin (1%), geniticin (400 µg/ml). Before experiments cells were split to single cells and allowed to settle on petri dishes. Effect of BIMU-8 on ion channels was determined through patch clamp experiments by using whole-cell configuration. BIMU-8 suppressed ionic currents both in $Na_v1.5$ and hERG channels in a dose dependent manner with IC_{50} of 1 µM (n = 5) and 56 nM (n = 4) respectively.

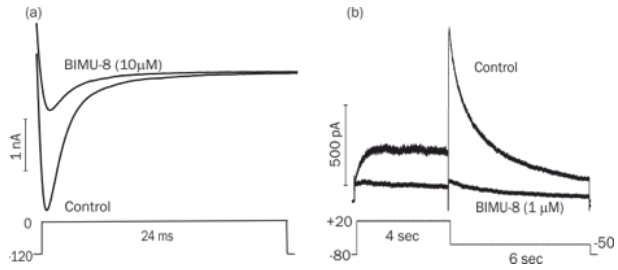


Fig. 1: Ionic currents suppression in both (a) $Na_v1.5$ and (b) hERG potassium channels

It is concluded from our preliminary experiments that BIMU-8 is a potent blocker of $Na_v1.5$ and hERG potassium channels, its applicability will be defined by further detailed experiments.

1. Nattel S, Carlsson L (2006) Nat Rev Drug Discov 5: 1034 - 1049.
2. Manzke T, Guenther U, Ponimaskin EG, Haller M, Dutschmann M, Schwarzacher S, Richter DW (2003) Science 301: 226 - 229.

Pre-clinical screening of novel biodegradable biomaterials for bone tissue engineering

Kampleitner, Carina[a], Richard, Christian[a], Hildebrand, Gerhard[b], Liefeith, Klaus[b], González, Constancio[c], Rodriguez-Cabello, José Carlos[c], Markus, Andrea[d], Dullin, Christian[d], Alves, Frauke[d], Hoffmann, Oskar[a]

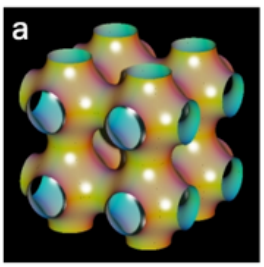
[a]Center of Pharmaceutical Science, Department of Pharmacology and Toxicology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria,[b]Institute for Bioprocessing and Analytical Measurement Techniques, Rosenhof, D-37308 Heilbad Heiligenstadt, Germany, [c]University of Valladolid, Campus de Miguel Delibes Paseo de Belén 11, Valladolid, Spain, [d]University Medical Center Göttingen, Robert-Koch Strasse 40, D-37075 Göttingen, Germany

Segmental bone loss caused by disease, trauma or tumor requires the use of bone graft substitutes for non-healing defects. Limited availability of autologous grafts and alloplastic materials combined with donor-site morbidity justify the design and development of alternative biomaterials. We sought to investigate the effects of novel biodegradable scaffold materials and injectable biogels designed to fill large lesions and improve bone healing.

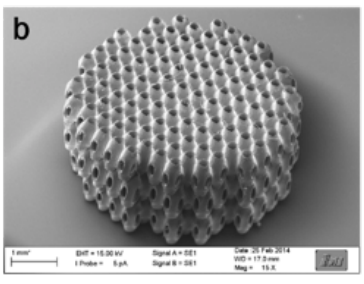
We tested 3 synthetic polymer scaffolds composed of lactide (LA), caprolactone (CL) and methacrylate (MA) with varying LA: CL ratio and %MA. Scaffold materials combined with thermosensitive elastin-like-recombinamer (ELR) gels containing either one growth factor (BMP2, BMP7) or a cell-adhesion sequence (RGD), and hydroxyapatite nanoparticles (HA NPs) were evaluated *in vitro* for mouse osteoblast (OB) and osteoclast (OC) function e.g., cell proliferation, alkaline phosphatase activity, mineralization and cell development, and *in vivo* for bone healing.

We observed that all scaffold materials induced cell attachment and proliferation, but only OBs cultured on LCM4 enabled mineralized matrix *in vitro*. OC development was supported by all scaffold materials. ELRs allowed OB differentiation and proliferation. However, growth factor containing ELRs did not enhance mineralization. Combined analysis of Quantum FX µCT and histology revealed *in vivo* cellular attachment and integration for all scaffold materials, suggesting an osteoconductive effect, although bone formation was only promoted for LCM3. Furthermore, osteogenesis was enhanced in combination with BMP2, BMP7 and nanoparticle containing ELRs.

These results illustrate that polymer composition and methacrylate content influence *in vitro* and *in vivo* OB and OC function. In conclusion, the combination of these biomaterials indicates an osteoinductive effect and provides new opportunities for repairing large bone lesions. (Supported by the European Seventh Framework Programme (NMP 2010.2-3-1) Grant agreement no. 263363).



(a) CAD 2x2x2 3-D solids of Schwarz's Primitive unit cells



(b) SEM image of a Ø 6 mm (height 2 mm) LCM scaffold composed of Schwarz´s Primitive unit cells (500 µm).

c

Scaffold material	LA : CL ratio	% MA
LCM3	8 LA: 2 CL	80%
LCM4	9 LA: 1 CL	90%
LCM6.1	9 LA: 1 CL	40%

(c) Scaffold formulations of biocompatible lactide, caprolactone scaffold.

P-25

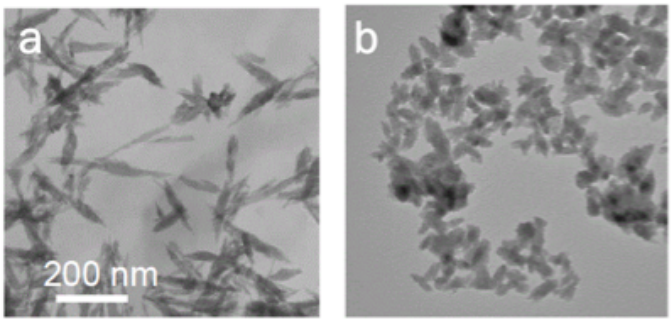
Effect of hydroxyapatite nanoparticles for regenerative medicine on primary mouse bone cells *in vitro*

Kanz, Stefan[a], Kampleitner, Carina[a], Espanol, Monserrat[b], Ginebra, Maria Pau[b], Hoffmann, Oskar[a]

[a]Center of Pharmaceutical Science, Department of Pharmacology and Toxicology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria, [b]Universitat Politecnica de Catalunya, C. Jordi Girona 31, E-08034 Barcelona, Spain

The use of nanoparticles (NPs) has great potential for monitoring and supporting bone tissue regeneration, due to their biocompatible, bioactive and osteoconductive nature. The cellular response to NPs depends on different parameters, such as size, shape, morphology, concentration and composition. We sought to investigate the biological effects of recently developed hydroxyapatite NPs on mouse osteoblasts (OB) and osteoclasts (OC) *in vitro*.
We compared the effect of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA;) and carbonate-substituted (10 wt%) apatite NPs for OB function and OC development. To determine OB differentiation, mouse calvarial-derived osteoblasts were cultured in the presence of various NP concentrations (20, 50, 100 or 250 µg/ml) and analysed for cell viability, alkaline phosphatase activity, collagen biosynthesis and non-collagenous protein secretion. OC development and function were evaluated using a mouse co-culture model, which contains OBs and OC precursors, and NPs (100 µg/ml) were added at different specific time points during OC differentiation and maturation.
We observed that both HA NPs types allowed proliferation, differentiation and matrix mineralization of primary mouse OBs. However, the growth of OBs, the differentiation capacity and the secretion of collagen and non-collagenous proteins were affected by NP composition in a dose-dependent manner, suggesting that a low dose exposure promotes OB differentiation and mineralization. Furthermore, the addition of carbonate-substituted HA NPs at early OC differentiation stages slightly decreased the number of multinucleated OCs, whereas addition at later time points did not affect OC development and resorption function.
In conclusion, these results reveal that NP composition and concentration can influence the cellular function of bone forming and bone resorbing cells *in vitro*. This finding should be taken into consideration for further *in vivo* application of NPs in a bone regenerative therapy.

(Supported by the European Seventh Framework Programme (NMP 2010.2-3-1) Grant agreement no. 263363)



TEM images of HA NPs.
(a) HA NPs (length: 139 ± 23 nm; width: 14 ± 6 nm) and
(b) Carbonate-substituted (10 wt%) HA NPs
(length: 99 ± 20 nm; width: 34 ± 5 nm)

Glycosylation-dependent activation of ENaC by the TNF lectin like domain derived peptide Solnatide

Waheed Shabbir[a,b], Susan Tzotzos[b], Minela Hasanovic[a], Mohammad Aufy[a], Anita Willam[a], Martin Kraihammer[a], Alexander Holzner[a], Hendrik Fischer[b], Helmut Pietschmann[b], Bernhard Fischer[b], Rudolf Lucas[c], Rosa Lemmens-Gruber[a]

[a]*Department of Pharmacology and Toxicology, Althanstraße 14, A-1090 Vienna, Austria*
[b]*APEPTICO GmbH, Mariahilfer Straße 136, A-1150 Vienna, Austria,*
[c]*Vascular Biology Center, Medical College of Georgia, 30912, GA, Augusta, United States*

Pulmonary edema is a life threatening condition, and a frequent complication of acute lung injury, characterized by loss of epithelial sodium channel (ENaC) function and cell surface expression in diseased epithelia. Solnatide, a TNF lectin like domain derived peptide, is being developed as a therapy for pulmonary edema, having recently completed phase 2a clinical trials. In this study we identify a glycosylation-dependent mechanism that preserves ENaC expression and function. Single- and multi-N-glycosylation site mutations were generated in α(N232,293,312,397,511Q)- and δ(N166,211,384Q)-subunits. Additionally, we have shown that the carboxy terminal of α-hENaC is essential for TNF lectin like domain interaction with ENaC [1]. Therefore, αL576X and αN232,293,312,397,511Q,L576X mutants were generated by deletion of the carboxy terminal of wild type and quintuple (N232,293,312,397,511Q) mutant α-αhENaC (Fig. 1). These constructs were co-expressed in HEK-293 cells with βγ-hENaC.

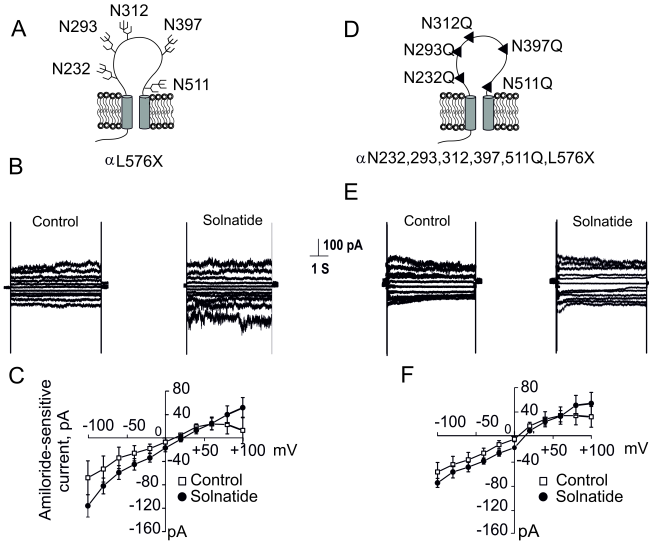


Figure 1:
A and D: model of membrane topology
B and E: whole cell current traces
C and F: IV-curves
(n=7-13)

In α(N232,293,312,397,511Q),L576Xβγ-hENaC, solnatide-induced activation, measured in the whole cell patch clamp mode, was abolished, and in αL576Xβγ-hENaC, it was attenuated (Fig. 1). Furthermore, in Western blot assays we found a significant increase (compared with control) of cell surface abundance of ENaC channel in WT following solnatide treatment and not in N to Q mutants. Taken together, our findings delineate a solnatide N-glycan dependent interaction leading to normalization of both sodium fluid absorption and ENaC cell surface expression in edematous alveoli to non-edematous levels.

1. Czikora I, Sridhar S, Gorshkov B et al. (2014) Am J Respir Crit Care Med. 190(5):522 – 32

P-27

Synthesis and in vitro characterization of novel valerenic acid analogues on $GABA_A$ receptors derived from a ligand-based pharmacophore model

Marco Stadler[a], Giovanna Parisi[b], Denise Luger[a], Thomas Seidel[b], Wolfgang Holzer[b], Vittorio Pace[b], Thierry Langer[b], Steffen Hering[a]

[a]Center of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [b]Center of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Aim:
Valerenic acid (VA), a major constituent from common *Valerian*, is a potent $\beta_{2/3}$ subunit-selective $GABA_A$ receptor modulator. VA's anxiolytic and anticonvulsive properties without concomitant sedation combined with a promising pharmacokinetic profile make this compound an interesting drug candidate [1, 2]. The aims of this study were the synthesis of small molecules based on the VA scaffold and their *in vitro* characterization.

Methods:
A pharmacophore model based on the known $\beta_{2/3}$ subunit-selective $GABA_A$ modulators loreclezole, etomidate and VA, suggested a series of novel, simplified VA analogues. Their effect on GABA-induced chloride currents (I_{GABA}) through $GABA_A$ receptors composed of $\alpha_1\beta_{1\text{-}3}\gamma_{2S}$ subunits expressed in *Xenopus laevis* oocytes was analysed by means of the two-microelectrode voltage clamp technique.

Results:
Efficacy of I_{GABA} enhancement by derivatives AR-013, AR-016 and SM-226-1 was comparable to that of VA, while slightly reduced potency was observed. The other studied compounds were either less efficacious or did not display significant potentiation of I_{GABA} at concentrations ≥30 µM.

Conclusion:
By using a ligand-based pharmacophore model, novel, simplified structures with $\beta_{2/3}$-selectivity comparable to that of VA were identified. These structures may serve as starting point for the development of novel, selective $GABA_A$ receptor modulators.

References
1. Hintersteiner J, Haider M, Luger D (2014) Eur J Pharmacol. 735: 123-31
2. Khom S, Strommer B, Ramharter J (2010) Br J Pharmacol. 161(1): 65-78

P-28

Mechanisms of Colon Cancer Entry Into Adjacent Stroma

Serena Stadler[a], Chi Huu Nguyen[a], Helmut Dolznig[b], Georg Krupitza[a]

[a] *Clinical Institute of Pathology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria*
[b] *Institute of Medical Genetics, Medical University of Vienna, Waehringerstrasse 10, A-1090 Vienna, Austria*

Colorectal Cancer (CRC) is the third most common cancer in western countries. Metastatic dissemination of primary tumor cells especially into liver and lungs is the prime cause of death in CRC patients. It is now well accepted that carcinogenesis is strongly supported by the tumor microenvironment, which consists mainly of cancer-associated fibroblasts (CAFs), endothelial cells and inflammatory cells that are embedded in the extracellular matrix (ECM). CAFs represent a major cellular component of the tumor microenvironment and are involved in CRC initiation, progression and metastasis. However, little is known about molecular interactions between colon cancer cells and CAFs in early stages of metastasis. Using a new 3D co-culture model we could demonstrate that colon cancer spheroids induce the retraction of CAFs leading to the formation of cell free areas, so called `circular chemorepellent induced defects ` (CCIDs) which resemble entry gates for the progressing tumor bulk into adjacent stroma. In order to identify underlying mechanisms we focused on the arachidonic acid metabolite 12(S)-HETE. 12-Lipoxygenase (12-LOX), the 12(S)-HETE producing enzyme, is overexpressed in various cancers and its metabolite is linked to cancer progression and metastasis. Indeed, inhibition of lipoxygenases in colon cancer–spheroids significantly decreases CCID-formation. Recently, the G-Protein coupled receptor, GPR31, was shown to be a high affinity receptor for 12(S)-HETE and its knock-down on the surface of CAFs clearly attenuated the formation CCIDs. Moreover, we could identify intracellular calcium - and ROCK1 signaling as driving pathways in this process. Pharmacological inhibition and knock-down of involved proteins and enzymes not only inhibit CCID formation but also prevent 12(S)-HETE-induced phosphorylation of the mobility proteins MLC2 and MYPT in CAFs, as shown by western blot analysis. Altogether, this insight into the complex tumor-stroma crosstalk provides new targets for therapeutic intervention and pharmacological drug development.

P-29

Comparative phytochemical analysis of four Globularia spp.

Maja Crkvenčić[a], Franz Bucar[b], Kroata Hazler Pilepić[a]

[a]*Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Botany, University of Zagreb, Schrottova 39, HR-10000 Zagreb, Croatia;* [b]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria*

Globularia alypum L. is a medicinal plant often used in folk medicine of Mediterranean countries for treatment of diabetes, digestive disorders and eczema [1,2]. Its biological activities can be connected to the presence of different phenolic and iridoid compounds [3]. The aim of the present study was to compare the phytochemical composition of three related *Globularia* species to that of *G. alypum*, in order to evaluate their pharmaceutical potential. Chemical composition of methanolic extracts of *G. alypum* L., *G. cordifolia* L., *G. meridionalis* (Podp.) O. Schwarz and *G. punctata* Lapeyr. obtained by boiling under reflux conditions was compared for the first time using LC-DAD-ESI-MS^n. Main components were identified by comparison of their retention time, UV and mass spectra (including MS^n up to MS^4) to those of reference compounds and/or to literature data.

Considering iridoid composition, catalpol and 10-O-esters of catalpol were characteristic for *G. alypum* and *G. punctata*, while 6-O-catalpol esters were observed only in *G. alypum*. High levels of globularin were observed in both species. Monomelittoside and its esters were characteristic for *G. cordifolia* and *G. meridionalis* extracts, with globularifolin present as a major compound. High amounts of asperuloside were observed in *G. cordifolia*, *G. meridionalis* and *G. punctata*, among which *G. punctata* contained high amounts of besperuloside, as well. All four species contained a large number of phenylethanoids, with verbascoside being the most abundant. Additionally, *G. punctata* contained high amounts of trichosanthoside A.

High amounts of bioactive compounds such as globularin [4], globularifolin [5] and verbascoside [6], observed in *G. cordifolia*, *G. meridionalis* and *G. punctata* suggest these species have potential for further exploitation.

1. Boudjelal A, Henchiri C, Sari M, Sarri D, Hendel N, Benkhaled A, Ruberto G (2013) J Ethnopharmacol 148: 395 – 402.
2. Jouad H, Haloui M, Rhiouani H, El Hilaly J, Eddouks M (2001) J Ethnopharmacol 77: 175 – 182.
3. Amessis-Ouchemoukh N, Abu-Reidah IM, Quirantes-Piné R, Rodríguez-Pérez C, Madani K, Fernández-Gutiérrez A, Segura-Carretero A (2014) Phytochem Anal 25: 389 – 398.
4. Merghache S, Zerriouh M, Merghache D, Tabti B, Djaziri R, Ghalem S (2013) J App Pharm Sci 3: 001- 007.
5. Sipahi H, Becker K, Gostner JM, Charehsaz M, Kirmizibekmez H, Schennach H, Aydin A, Fuchs D (2014) Fitoterapia 92: 85 – 92.
6. Alipieva K, Korkina L, Orhan IE, Georgiev MI (2014) Biotechnol Adv 32: 1065-1076.

P-30

Analysis of volatile constituents of Salvia pomifera and S. fruticosa

Antonios Koutsoulas[a], Andrea Böszörményi[b], Jaroslav Tóth[a], Erzsébet Háznagy-Radnai[c], Szilvia Czigle[a]

[a]Comenius University in Bratislava, Faculty of Pharmacy, Department of Pharmacognosy and Botany, Odbojárov 10, SK-83232 Bratislava, Slovakia; e-mail: czigle@fpharm.uniba.sk; [b]Semmelweis University, Faculty of Pharmacy, Institute of Pharmacognosy, Üllői 26, H-1085 Budapest, Hungary; [c]University of Szeged, Faculty of Pharmacy, Department of Pharmacognosy, Eötvös 6, H-6720 Szeged, Hungary

The genus *Salvia* L. was known in Ancient Greece already, mentioned even by both Theophrastus and Dioscurides Pedanius. *Salvia pomifera* L. and *Salvia fruticosa* Mill. (Lamiaceae) are East-Mediterranean species, restricted to Greece and to Asia Minor and typical to the island of Crete. A long tradition of use is found in Greece, where the plants are valued for medicinal, beauty, and culinary use.

The EMA-HMPC is currently working on a new Community Herbal Monograph (Salviae fruticosae herba).

The aim of this study was to analyse the volatile compounds in Salviae pomiferae herba and Salviae fruticosae herba by SPME GC-MS. The plant material originated from Crete. The harvests were carried out in June 2013, during the optimal phenophase of early blooming.

We identified 29 components in Salviae pomiferae herba, i.e. 98.46 % of the volatile components. The components identified in highest percentage were: -thujone (23.54 %), β-thujone (23.20 %), and β-caryophyllene (10.8 %). Nine components were identified in Salviae fruticosae herba, i.e. 98.5 % of the volatile components. These volatile components were identified in highest percentage: 1,8–cineole (40.74 %), camphor (7.8 %), and (-)–pinene (6.17 %).

The herbal drug Salviae fruticosae herba contained only 6.04 % of β-thujone, and 1.76 % of -thujone, compared to high amounts of these compounds in Salviae pomiferae herba.

P-31

Honokiol derivatives as RXRα modulators

Latkolik SL[a], Fakhrudin N[a,b], Rycek L[c],Ladurner A[a], Malainer C[a], Heiss EH[a], Schwaiger S[d], Stuppner H[4], Mihovilovic MD[c], Schuehly W[e], Atanasov AG[a], Dirsch VM[a]

a Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; b Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, 55281 Yogyakarta, Indonesia; c Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163-OC,1060 Vienna, Austria; d Institute of Pharmacy, University of Innsbruck, Innrain 80 - 82/IV, 6020 Innsbruck, Austria; e Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, A-8010 Graz, Austria

Retinoid X Receptors (RXRs) are nuclear receptors displaying a variety of biological functions. They regulate physiological and developmental processes by acting as signal integrators to control the transcription of target genes.[1]
The ability of ligands to modulate RXR nuclear function either as agonist or antagonist is due to the ligand-receptor complex formation, the ability of RXRs to heterodimerize with other nuclear receptors or to form homodimeric receptor- complexes, as well as the ability of the formed ligand-receptor complex to differentially recruit diverse co-activators and co-repressors to the respective target gene promoters. Some small molecule ligands of RXR excert anticancer activity and have a potential for the treatment of metabolic diseases but their usage is limited due to toxicity and unfavorable tissue and receptor subtype selectivity.[2] The aim of this work is to identify selective small-molecule ligands for RXR and to further characterize their binding modalities as well as their biological function. Honokiol, a biphenylic neolignan that was initially isolated from the bark of Magnolia officinalis, served as lead structure.[3] It has been shown that honokiol promotes RXRα-, as well as PPARγ-dependent luciferase gene expression in human embryonic kidney cells (HEK293).[4] By using receptor specific luciferase-based testing models, we identified 5 honokiol derivatives that selectively transactivate RXRα-dependent but not LXRα-, LXRβ-, PPARγ- and FXR-dependent luciferase gene expression in a dose-dependent manner. The calculated EC_{50} values for RXRα activation indicate a high potency of action of these derivatives and range from 330 to 850 nM. The ability of transactivating RXRα-dependent genes could be reversed by co-treatment with the RXRα antagonist HX531. Furthermore we confirmed the agnositic activity using a mammalian one-hybrid assay with a Gal4-RXRα ligand-binding domain.[5] Therefore, based on this data, we conclude that the we identified new RXRα agonists that act selectively on this nuclear receptor.[6]

1. 1 Evans, R. M. & Mangelsdorf, D. J. Nuclear Receptors, RXR, and the Big Bang. Cell 157, 255-266, doi: 10.1016/j.cell.2014.03.012 (2014).
2. de Lera, A. R., Bourguet, W., Altucci, L. & Gronemeyer, H. Design of selective nuclear receptor modulators: RAR and RXR as a case study. Nat Rev Drug Discov 6, 811-820, doi:10.1038/nrd2398 (2007).
3. Kumar, A., Kumar Singh, U. & Chaudhary, A. Honokiol analogs: a novel class of anticancer agents targeting cell signaling pathways and other bioactivities. Future Med Chem 5, 809-829, doi:10.4155/fmc.13.32 (2013).
4. Atanasov, A. G. et al. Honokiol: a non-adipogenic PPARgamma agonist from nature. Biochim Biophys Acta 1830, 4813-4819, doi:10.1016/j.bbagen.2013.06.021 (2013).
5. Umesono, K., Murakami, K. K., Thompson, C. C. & Evans, R. M. Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. Cell 65, 1255-1266 (1991).
6. Lefebvre, P., Benomar, Y. & Staels, B. Retinoid X receptors: common heterodimerization partners with distinct functions.

Secondary metabolites of the marine Actinomycete Williamsia maris

Andrea Lubich[a], Martin Zehl[a,b], Hanspeter Kählig[c], Liselotte Kenna

[1] *Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria*
[2] *Department of Pharmaceutical Chemistry, University of Vienna, Althanstraße 14, 1090 Vienna, Austria*
[3] *Institute of Organic Chemistry, University of Vienna, Währinger Straße 38, 1090 Vienna, Austria*

The marine environment is recognized as the space with the highest biodiversity on earth and the richest source for new lead structures in drug development. In this context, microbes are supposed to produce the largest number and variety of marine secondary metabolites [1, 2]. Among microorganisms, bacteria of the order Actinomycetales are most promising for the elucidation of new structures as 45% of all previously discovered microbial secondary metabolites derive from this order [3]. This fact prompted us to focus our search for new natural products on marine Actinomycetales.

In the project, the scarcely examined strain *Williamsia maris,* which was initially isolated from the Sea of Japan, was obtained from DMSZ (Braunschweig, Germany) and cultivated in a GYM-medium (0.4% Glucose, 0.4% yeast extract, 1% malt extract) for 14 days [4]. By centrifugation the cells were removed and the resin Amberlite XAD-16 was added to the fermentation broth to adsorb released metabolites. After two days of incubation the adsorbate was eluted from the resin with acetone to gain 1.5 gram of dried crude “XAD-extract”. This extract was fractionated by SPE on RP-18 cartridges into 18 fractions. Subsequent size-exclusion chromatography led to the isolation of three compounds. Their structures were determined by high-resolution MS and NMR spectros-copy as lumichrome, indole-3-carbaldehyde and a 3-O-methyl mannose polysaccharide. LC-MS analysis of another fraction of the XAD-extract pointed to the presence of two cyclic dipeptides with masses and fragmentation patterns correlating with those of cyclo(Ile-Pro) and cyclo(Leu-Pro), respectively [5]. All five substances are reported in the strain *Williamsia maris* for the first time.

1. Abdelmohsen UR, Bayer K, Hentschel U (2014) Nat Prod Rep 31: 381-399.
2. Gerwick WH Fenner AM (2013) Microb Ecol 65: 800-6.
3. Subramani R Aalbersberg W (2012) Microbiol Res 167: 571-580.
4. Stach JE et al. (2004) Int J Syst Evol Microbiol 54: 191-4.
5. Chen Y-H, Liou S-E, Chen C-C, (2004) Eur Food Res Technol. 218: 589-597.

P-33

Polyphenol Constituents Responsible for Antiaggregatory Activity of Propolis

Mirza Bojic, Andrea Antolic, Hrvoje Rimac

[a]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*

Propolis presents one of the richest sources of polyphenols and has numerous biological activities that include antibacterial, antifungal, antiviral, antineoplastic, hepatoprotective, immunomodulating, and anti-inflammatory, etc. As we have previously shown, flavonoids influence platelet aggregation[1], thus the goal of this study was to determine phenolic acids and flavonoids in propolis that contribute the most to the antiaggregatory activity of propolis.
Twenty-two ethanolic extracts were analyzed by HPLC equipped with diode array detector. Separation of polyphenols analyzed was performed on a Agilent Zorbax SBC18 column (250mm×4.6mm, particle size 5µm), using mobile phases: A-water/methanol/formic acid (93:5:2) and B-water/methanol/formic acid (3:95:2) with the following timetable (t/min, %B): (0,20), (10,40), (35,50), (47,50), (70,80), (80,20). Chromatograms were recorded at 270, 290, 320 and 350 nm. Platelet aggregation was analyzed in whole blood utilizing an impedance analyzer. The results of aggregation assay were expressed as the lowest concentration of flavonoid that caused a statistically significant reduction of aggregation when compared to the untreated samples.
Multiple linear regression analysis and random forest was used to assess the constituents of propolis that contribute the most to the antiaggregatory activity of propolis. The most prominent constituents were total phenolic acids and content of flavonoid apigenin.

1. Bojic M, Debeljak Z., Tomicic M, Medic-Saric M (2011), Nut. J. 10: 73.

P-34

Combination of a QuEChERS-based extraction protocol with a fast and selective UHPLC-QTOF-MS assay for the detection and quantification of Metarhizium brunneum metabolites from honey samples

Judith Taibon[a,b], Sonja Sturm[a], Christoph Seger[a], Hermann Strasser[b], Hermann Stuppner[a]

[a]*Institute of Pharmacy, Department of Pharmacognosy, University of Innsbruck, CCB – Centrum of Chemistry and Biomedicine, Innrain 80/82, A–6020 Innsbruck, Austria;* [b]*Institute of Microbiology, University of Innsbruck, Technikerstraße 25, A-6020 Innsbruck, Austria*

Varroa destructor Anderson and Trueman (2000) is an ectoparasitic mite which is currently one of the most serious threats for apiculture [1,2]. Since the honey bee *A. mellifera* is crucial not only for honey production but also for crop pollination varroa control is of great interest [1]. A promising alternative strategy to synthetic acaricides or organic acids could therefore be the use of entomopathogenic fungi e.g. *Metarhizium* spp. or *Beauveria* spp. [3,4]. Although *M. brunneum* plays an important role as a biological control agent (BCA) [5] there are still concerns that its bioactive secondary metabolites, so called destruxins (dtxs), imparts risks to humans and the environment. Hence, the availability of analytical assays for destruxin trace analysis is of great necessity.

Therefore, a previously established QuEChERS-based extraction protocol in combination with a fast and selective UHPLC-QTOF-MS assay has been extended for the detection and quantification of destruxins from honey [6]. The internal standard fortified assay showed satisfying assay precision and accuracy and allowed quantification of destruxin A and B down to ppb range with limits of detection (LOD) and quantification (LOQ) of less than 1.0 ppb and 2.3 ppb, respectively. Destruxin E showed a distinctive loss of recovery and was therefore excluded from further quantitative analysis.

The developed assay was used to screen honey samples obtained from a trial where *M. brunneum*, strain BIPESCO 5, was used to control the ectoparasitic mite *Varroa destructor* in honey bee colonies. From the original sixteen samples seven controls and seven treatment samples were analysed with the result that no traces of destruxins were found in any sample.

1. Kanga LHB, Adamczyk J, Patt J, Gracia C, Cascino J (2010) Development of a user-friendly delivery method for the fungus *Metarhizium anisopliae* to control the ectoparasitic mite *Varroa destructor* in honey bee, *Apis mellifera*, colonies, Exp. Appl. Acarol. 52: 327–342.
2. García-Fernández P, Santiago-Álvarez C, Quesada-Moraga E (2008) Pathogenicity and thermal biology of mitosporic fungi as potential microbial control agents of *Varroa destructor* (Acari: Mesostigmata), an ectoparasitic mite of honey bee, *Apis mellifera* (Hymenoptera: Apidae), Apidologie 39: 662–673.
3. Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*, J. Invertebr. Pathol. 103: 96–119.
4. Hamiduzzaman MM, Sinia A, Guzman-Novoa E, Goodwin PH, (2012) Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, *Varroa destructor*, and their effect on the immune response of honey bees (*Apis mellifera* L.), J. Invertebr. Pathol. 111: 237–243.
5. Zimmermann G (2007) Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*, Biocontrol. Sci. Technol. 17:879–920.
6. Taibon J, Sturm S, Seger C, Strasser H,Stuppner H (2015) Quantitative assessment of destruxins from strawberry and maize in the lower parts per billion range: combination of a QuEChERS-based extraction protocol with a fast and selective UHPLC-QTOF-MS assay, J. Agric. Food Chem. 63: 5707–5713.

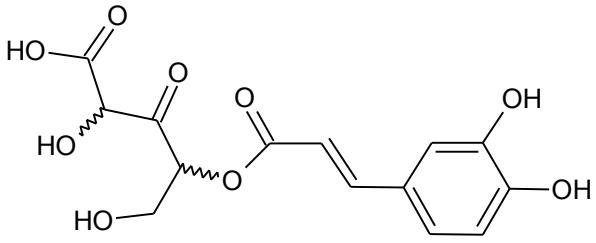
P-35

Justicia secunda VAHL (Acanthaceae): Phytochemical Investigations and α-Glucosidase Inhibition

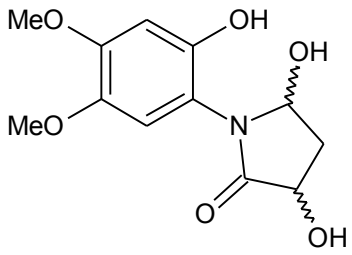
Barbara A. Theiler[a], Stefanie Istvanits[a], Martin Zehl[a,b], Ernst Urban[b], Laurence Marcourt[c], Lugardo O. Espinoza-Caisa[d], Sabine Glasl[a]

[a]*Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria*
[b]*Department of Pharmaceutical Chemistry, University of Vienna, Althanstraße 14, 1090 Vienna, Austria*
[c]*Department of Phytochemistry and Bioactive Natural Products, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland*
[d]*Corporación Verde Azul, Santa Prisca OE 3-17, 5to Piso, Ofic 27, 170150 Quito, Ecuador*

Justicia secunda VAHL belongs to the family of Acanthaceae and is a barely examined species. In Ecuador, the leaves of *J. secunda* are applied in ethnomedicine against indications related to diabetes [1]. The aim of this study was to screen *J. secunda* for its potential α-glucosidase inhibiting effect performing a bioautographic HPTLC assay [2]. A methanol extract of leaves was first partitioned successively between solvents of increasing polarity (light petroleum, CH_2Cl_2, BuOH, H_2O). The aqueous fraction showed distinct activity. It was subjected to column chromatography on polystyrene for further fractionation and sugar removal, yielding two subfractions with α-glucosidase inhibiting effects. For final isolation and characterization of pure active compounds, preparative HPTLC and semipreparative HPLC on RP-18e were conducted. MS and extensive NMR measurements led to the structure elucidation of the unknown diastereomers 4RS-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-2RS,5-dihydroxy-3-oxopentanoic acid (1) and the recently published diastereomers secundarellone B and C (2) [3].



1



2

References

1. Personal communication with “Corporación Verde Azul” in Ecuador.
2. Simões-Pires CA, Hmicha B, Marston A, Hostettmann K (2009) Phytochem Anal. A TLC bioautographic method for the detection of α- and β-glucosidase inhibitors in plant extracts. 6: 511-515.
3. Theiler BA, Revoltella S, Zehl M, Dangl C, Espinoza Caisa LO, König J, Winkler J, Urban E, Glasl S (2014) Phytochem Lett. Secundarellone A, B, and C from the leaves of *Justicia secunda* VAHL. 10: cxxix-cxxxii.

Evodiamine Induces Cholesterol Efflux through Inhibiting ABCA1 Protein Degradation

Limei Wang[a], Irena D. Ignatova[b], Veronika Palme[a], Elke H. Heiss[a], Herbert Stangl[c], Ira G. Schulman[b], Verena M. Dirsch[a], Atanas G. Atanasov[a]

[a]Department of Pharmacognosy, University of Vienna, Vienna, Austria; [b]Department of Pharmacology, University of Virginia Health System, Charlottesville, VA, USA; [c]Institute of Medical Chemistry, Center for Pathobiochemistry and Genetics, Medical University of Vienna, Vienna, Austria

Cholesterol efflux (ChE), the initial step of reverse cholesterol transport (RCT), comprises the export of cholesterol from macrophages. Increased ChE and reduced foam cell formation are regarded to have an anti-atherosclerotic effect. Thus, identification and characterization of molecules that stimulate ChE might be of pharmacological relevance. In this study, we investigated the influence of evodiamine, an indoloquinazoline alkaloid isolated from the fruits of *Evodia rutaecarpa* [1], on macrophage ChE and further explored its mechanism.
Evodiamine dose-dependently increases ChE in THP 1 macrophages without affecting cell viability as determined by resazurin conversion [2]. Western blot analyses show that the protein level of ATP-binding cassette transporter A1 (ABCA1) increases in response to evodiamine treatment, while the protein levels of ABCG1 and scavenger receptor class B member 1 (SR-BI) remain unaffected. These three proteins are key membrane transporters contributing to ChE [3]. Evodiamine exerts neither an influence on ABCA1 mRNA levels as determined by quantitative real time PCR nor on the human ABCA1 promotor activity as revealed by a luciferase reporter assay. In contrast, evodiamine significantly inhibits the degradation of ABCA1 as evident by an increased half-life of the protein in the presence of cycloheximide, an inhibitor of *de novo* protein biosynthesis.
Taken together, Evodiamine promoted ChE in THP 1 macrophages by preventing ABCA1 from degradation. Our results indicate a novel anti-atherosclerotic property of evodiamine.

Acknowledgements: This work was supported by the Austrian Science Fund (FWF) project P25971-B23 ('Improved cholesterol efflux by natural products'), and by the Vienna Anniversary Foundation for Higher Education (Hochschuljubiläumsstiftung der Stadt Wien) project H-297332/2014 ('Metabolomics-assisted dissection of molecular mechanisms underlying the action of natural products increasing macrophage cholesterol efflux').

References:
1. Adams M, Mahringer A, Kunert O, Fricker G, Efferth T, Bauer R (2007) Planta med 73(15):1554-1557.
2. Ladurner A, Atanasov AG, Heiss EH, Baumgartner L, Schwaiger S, Rollinger JM, Stuppner H, and Dirsch V (2012) Biochem pharmacol 84: 804-812.
3. Duffy D, Rader D (2006) Circulation 113.8: 1140-1150.

P-37

Phenolic metabolites of *Eriosema laurentii* De Wild.

Benjamin Sylvin Ateba[a,b], Dieudonné Njamen[a], Martin Zehl[b], Claus Gatterer[b], Hanspeter Kählig[c], Liselotte Krenn[b]

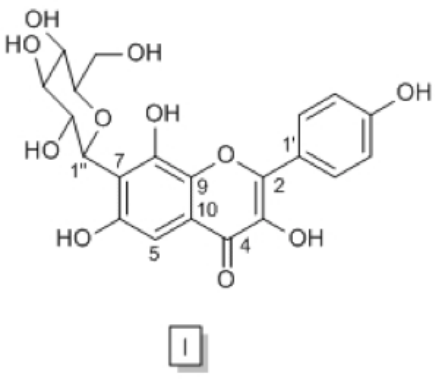
[a]*Department of Animal Biology and Physiology, University of Yaounde I, Box 812 Yaounde, Cameroon* [b]*Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria*
[c]*Institute of Organic Chemistry, University of Vienna,Währingerstr.38, 1090 Vienna, Austria*

The African plant *Eriosema laurentii* De Wild. is widely used in Cameroon for the treatment of infertility and gynaecological and menopausal complaints. Pharmacological studies have proven the potential of an extract from this plant for the treatment of menopausal complaints and identified isoflavones as active compounds [1,2]. In continuation of the phytochemical investigation, two new natural phenolics with a hitherto unknown substitution pattern, namely 3,4',6,8-tetrahydroxyflavon-7-C-glucoside (I) and 3,4',6,8-tetrahydroxy-flavanon-7-C-glucoside (II), were isolated from the methanolic extract and structurally elucidated.

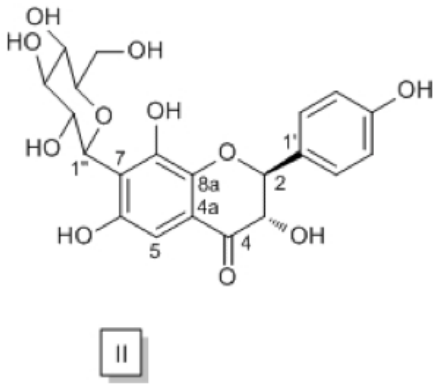
The isolation was performed by liquid-liquid partition of the extract, column chromatography on silica gel and Sephadex LH-20 and by high performance counter current chromatography of the fractions. For dereplication thin layer chromatography, high performance liquid chromatography and liquid chromatography-mass spectrometry were applied. Mass spectrometry and one- and two-dimensional nuclear magnetic resonance techniques were used for the structure elucidation of the new compounds.

Besides the two new substances by these methods several known phenolic constituents were identified as 2"-O--rhamnosyl-6-C-fucosyl-3´-methoxy-luteolin, 2'-hydroxygenistein-7-O-glucosid, genistein-8-C-glucosid, syringaresinol, 2,6-dihydroxybenzoic acid and 3,4-dihydroxybenzoic acid, all for the first time in *E. laurentii*.

1. Ateba SB, Njamen D, Medjakovic S, Zehl M, Kaehlig H, Jungbauer A, Krenn L. (2014) BMC Comp Altern Med 14: 294-303
2. Ateba SB, Njamen D, Medjakovic S, Hobiger S, Mbanya JC, Jungbauer A, Krenn L (2013) J Ethnopharmacol 150: 298-307



I



II

Effect of plasma from high- and low- serum unconjugated bilirubin (UCB) individuals on cholesterol efflux

Dongdong Wang[a, b], Anela Tosevska[a, c], Limei Wang[a], Karl-Heinz Wagner[c, d], Verena M. Dirsch[a], Atanas G. Atanasov[a, *]

[a]Department of Pharmacognosy, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [b]Department of Pharmacognosy, West China College of Pharmacy, Sichuan University, No. 17, Duan 3, Renmin Nan Road, 610041 Chengdu, China; [c]Research Platform Active Ageing, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [d]Department of Nutritional Sciences, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Aim: Mildly elevated bilirubin levels, a characteristic of Gilbert Syndrome (GS), have been inversely correlated with cardiovascular disease. Protection of lipids, proteins and other macromolecules from oxidation by bilirubin is the most commonly accepted mechanism of cardiovascular protection [1]. A critical step in atherosclerosis pathogenesis is the formation of cholesterol-enriched pro-atherogenic foam cells. Therefore, increased macrophage cholesterol efflux is expected to result in an overall anti-atherosclerotic effect. Data, however, how high serum levels of unconjugated bilirubin (UCB) might affect macrophage cholesterol efflux are lacking. Thus, aim of this study was to investigate the effect of plasma from high- and low-serum UCB individuals on cholesterol efflux.

Methods: In this study, THP-1-derived macrophages were differentiated by PMA treatment and then labelled with [^{3}H]cholesterol. Cholesterol efflux was assessed in presence of human plasma from 120 individuals for 4 h. The subjects were divided into two age- and gender-matched groups, with high and low serum UCB with a cut-off point at 17.1 µM. A paired *t*-test was performed to analyse the data for statistical significance.

Results: The percentage of cholesterol efflux mediated by serum from high- and low-UCB individuals was 5.454% and 5.827%, respectively. Individuals with higher serum UCB showed significantly lower cholesterol efflux capacity ($p < 0.001$), even after correction for Apo-A1 or HDL levels in plasma.

Discussion and conclusion: A number of studies have shown that risk of mortality from cardiovascular disease is remarkably reduced in GS individuals. This protection may be explained by bilirubin's ability to protect blood lipids and LDL from oxidation [1]. Some studies showed that the individuals with GS had significantly reduced levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG), oxidized low density lipoprotein (oxLDL), very low-density lipoprotein (VLDL), small dense low-density lipoprotein (sd-LDL), and elevated HDL/LDL ratios in plasma [2]. However, our results showed that plasma with higher bilirubin levels, as found in GS, do not contribute to higher cholesterol efflux from macrophages, and even had a reverse effect. This suggests that there might be different pathways involved in the cardiovascular protection by increased plasma bilirubin.

1. Wallner M, Marculescu R, Doberer D, Wolzt M, Wagner O, Vitek L, Bulmer AC, Wagner KH. (2013) Clin Sci, 125(5): 257-264.
2. Bulmer AC, Verkade HJ, Wagner KH. (2013) Prog Lipid Res, 52(2): 193-205.

P-39

A Novel Approach to Azido-substituted Cyclodextrins

Maryam Amiri[1], Supatta Midpanon[1], Werner Praznik[1], Wolfgang Holzer[2], Helmut Viernstein[1], Frank M. Unger[1]

[1]Faculty of Life Sciences, Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [2]Faculty of Life Sciences, Department of Pharmaceutical Chemistry, Division of Drug Synthesis, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.

Introduction: Cyclodextrin glucosyltransferase (CGTase; EC 2.4.1.19) from *Bacillus macerans* is known for conventionally producing cyclodextrins (CDs) from starch through its capability of catalyzing reactions by cyclization [1]. In certain water-organic solvent systems and specific conditions, low-mol-wt maltose has been used as a substitute for starch in the synthesis of oligosaccharides and CDs [2].

Aim: The enzymatic synthesis of oligosaccharides and CDs using maltose instead of starch offers the possibility to introduce different functional groups (such as azido-groups) into a CD ring, affording precursors for amino-derivatives of the same with enhanced properties in drug delivery applications. The chemically synthesized maltose derivatives 2-azido-, 6-azido- and 6´-azidomaltose [3] have been studied as alternative substrates for CGTase.

Methods: CGTase-catalyzed reactions were performed by placing maltose or maltose analogues (0.15 molar) into 44% (v/v) cyclohexane in water. CGTase at a concentration of 300 U/g maltose or maltose analogue was added while constantly stirring with a magnetic stirrer (400 rpm) at 7°C. The reactions were monitored using thin layer chromatography (TLC) and subjected to RPHPLC for CD- and HPAEC-PAD for oligosaccharide analysis.

Results: The maximum yield of β-CD from maltose was reached after 72h. Regarding the azido-substituted maltose derivatives the production of only oligosaccharides was documented during the reaction of 2-azido- and 6´-azidomaltose while CD-production was observed exclusively with 6-azidomaltose. It is assumed that the reason might be the favored reaction of the enzyme with the non-reducing moiety and the anomeric region of the reducing unit.

Conclusion: 6-Azido-6-deoxy-maltose is a good substrate for CGTase regarding CD-production. The sterically less favorable 2-azido- and 6´-azidomaltoses were still converted. The capability of the azido-group to undergo transformations and be tolerated by CGTase offers for azido sugars a wide spectrum of technical, medical and pharmaceutical applications such as diagnostics and novel excipients produced in an environment-friendly way.

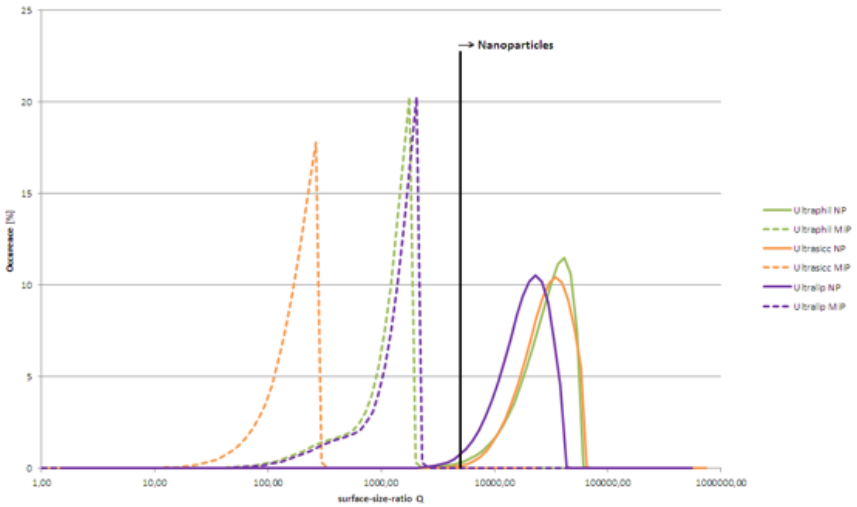
1. Ohnishi M, Ota U, Tonomura B, Kubota M (1992) Carbohydr. Res. 227: 285-291.
2. Morita T, Yoshida N, Karube I (1996) Appl. Biochem. Biotech. 56: 311-324.
3. Midpanon S (2015) Unpublished Dissertation, University of Vienna, PTB.

Analysis of nanoparticles in different semi-solid systems

Astrid Pany[a], Corinna Nagelreiter[a], Claudia Valenta[a,b]

[a] *Center of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria ;*
[b] *Research Platform "Characterisation of Drug Delivery Systems on Skin and Investigations of Involved Mechanisms", University of Vienna, Althanstraße 14, A-1090 Vienna, Austria*

Due to the EU Cosmetics Directive 1223/2009 nanoparticles have to be declared "nano" since 2013. Therefore, a method using laser diffraction for assessment was developed by Nagelreiter and Valenta [1]. The latest enhancement of this method allows analysis of not only O/W and W/O formulations, but also hydrophobic ointments even faster and more cost-effective.
TiO_2 nanoparticles and $MgCO_3$ microparticles were incorporated in various semi-solid systems. While O/W and amphiphilic formulations were converted into milks by dilution with distilled water, W/O formulations and hydrophobic ointments were heated and stirred with Tween 80 before adding water. The resulting milk was broken by addition of 10 M NaOH solution and particles were separated by centrifugation at 12000 rpm. After re-suspending the sediment with fresh water, the suspension was extracted with n-hexane to remove any remnants of fat. This step was repeated until solely particles were present after centrifugation.
In order to assess the size distribution, the Mastersizer 3000 (Malvern, UK) utilising laser diffraction was employed. Since nanoparticles tend to agglomerate in hydrophilic environment, the surface-size-ratio Q [1] was applied to distinguish agglomerated nanoparticles from microparticles.



Using this technique, nanoparticles showing their Q maxima above the boundary limit could clearly be differentiated from microparticles as pictured in Figure 1.

1. Nagelreiter, C., Valenta C., Size analysis of nanoparticles in commercial O/W sunscreens. Int. J. Pharm., 456, 517-519 (2013)

P-41

Cajanus cajan – a source for PPARγ activators leading to anti-inflammatory and anti-cancerogenic effects

Roswitha Schuster, Helmut Viernstein, Monika Mueller

Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090, Vienna, Austria

Inflammation and cell differentiation play a critical role in various diseases (eg: rheumatoid arthritis, atherosclerosis, diabetes, asthma, cancer, struma, etc.). Conventional treatments exert excellent curative effects, but show several serious side effects. Natural remedies, which show complementary bioactivity, may be effective in alternative prophylaxis and treatment.

We focus on role of peroxisome proliferator-activated receptor gamma (PPARγ) as potential therapeutic target of *C. cajan* and its isolated compounds for the treatment of cancer, inflammation and inflammation-related disorders. We also elucidate the anti-inflammatory potential of *C. cajan* and its bioactive compounds, as well as the cytotoxicity on the human cervical adenocarcinoma cell line HeLa, the human colorectal adenocarcinoma cell line CaCo-2 and the human breast adenocarcinoma MCF-7 cell line. Furthermore the main active compounds of *C. cajan* are isolated and identified.

C. cajan was separated by preparative high performance liquid chromatography (HPLC) to obtain single fractions. The Interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrose factor alpha (TNF-α) production of lipopolysaccharide stimulated macrophages was quantified with the help of an enzyme linked immonosorbent assay (ELISA), testing *C. cajan* extract and its isolated compounds in various concentrations. Cyclooxigenase-2 (COX-2) and nitric oxide synthase (iNOS) activation was determined by performing a western blot. Anti-carcinogenetic activity was determined using a MTT-assay. A PPARγ transactivation assay was performed to assess the potential of *C. cajan* and its isolated compounds as a PPAR activator.

The concentration of secreted IL-6 was significantly reduced in response to treatment with *C. cajan* and most of its isolated compounds. No significant inhibition was found on IL-10 secretions, but a slightly reduction of TNF-α was determined for 2 isolated fractions. The expression of COX-2 and iNOS was significantly reduced by 2 isolated fractions and moderately reduced by *C. cajan* extract and 1 isolated fraction. There was a great variation of anti-carcinogenetic effect of *C. cajan* and its isolated compounds in the three cancer cell lines, but more than 80% showed significant toxic effect. *C. cajan* exerts very good PPARγ activity, 3 isolated compounds show high transactivation values.

In conclusion, this study revealed that *C. cajan* and its isolated compounds show good anti-inflammatory and anti-carcinogenetic effects as well as PPARγ activity *in vitro*. Based on this data, *C. cajan* provides excellent beneficial medicinal attributes and may be used as potential food or pharmaceutical supplement.

P-42

Alcoholic Beverages and Medications: A safety Concern?

Simone Schrank[a], Eva Roblegg[a,b]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz, Austria, Austria;* [b]*Research Center Pharmaceutical Engineering GmbH, Graz, Austria*

The concomitant intake of alcoholic beverages and modified-release oral dosage forms poses a serious safety concern for humans. This is specifically true for drugs that show a narrow therapeutic index [1], since alcohol has the potential to break down the release regulating barriers, which are responsible to regulate the drug dissolution over extended time periods in aqueous media. Consequently, a significant fraction of the drug is released within a short time period, thus, alcohol induced dose dumping (ADD) occurs. In previous studies it was shown that hot melt-extruded pellets based on calcium stearate (CaSt) result in a retarded formulation, which is not affected by the addition of alcohol [2,3].

The present study aims at developing an alcohol resistant multi-particulate dosage form via wet extrusion/spheronization using CaSt and codeine phosphate (COP). As the drying conditions affect the microstructure and thus, drug release rates [4, 5, 6], the drying process (i.e., tray or fluid bed drying at temperatures ranging between 30 and 50 °C) was adjusted to yield modified release. In order to achieve ADD resistance, additives that are insoluble in ethanol (i.e., xanthan gum, guar gum and TiO_2) were incorporated.

The pellet microstructure was a function of both, the drying technique and the drying temperature. The free surface area increased with increasing temperature as CaSt shrinkage was less pronounced. For drying temperatures of 30 °C and 40 °C fluid bed drying yielded lower specific surface areas compared to tray drying. In contrast, pellets dried at 50 °C showed similar surface areas independent upon the drying technique. Interestingly, the COP release in acidic medium (gastric conditions) was higher for pellets dried in the fluid bed (30 and 40 °C) despite lower specific surface areas. However, when the pH of the dissolution medium was increased to mimic intestinal conditions, COP release was higher for tray dried pellets. Overall, drying in the fluid-bed apparatus at 30 °C yielded modified COP release over 8 hours due to the lowest specific surface area. All pellets were ADD resistant in acidic medium comprising 20% ethanol. However, when the ethanol content was increased to 40%, the fraction of COP released was doubled due to CaSt swelling resulting in an increased surface area accessible to the dissolution medium. Hence, additives that do not interact with ethanol were incorporated and the pellets were dried in the fluid bed apparatus at 30 °C. The addition of xanthan yielded extrudates that were not suitable for spheronization. When guar gum was added the modified release characteristics were lost, as guar gum swells in aqueous media and consequently, elevated the COP release rates. The addition of 10% TiO_2 only slightly increased the COP release rates – hence, still providing modified release – and prevented ADD in acidic medium containing up to 40% ethanol. Summarizing, we showed that CaSt is a promising matrix system for modified release. ADD resistance of these systems was achieved by the incorporation of TiO_2 and not via film coating, which is currently the only way to ensure ADD resistance of pellets.

1. Jedinger N, Khinast J, Roblegg E (2014) Eur J Pharm Biopharm, (2014) 87(2):217-26.
2. Roblegg E, Jäger E, Hodzic A, Koscher G, Mohr S, Zimmer A (2011) Eur J Pharm Biopharm 79:635-645.
3. Jedinger N, Schrank S, Mohr S, Feichtinger A, Khinast J, Roblegg E., Eur J Pharm Biopharm 92:83-95.
4. Schrank S, Hodzic A, Zimmer A, Glasser B, Khinast J, Roblegg E (2012) AAPS PharmSciTech 13(2): 686-698.
5. Schrank S, Kann B, Windbergs M, Glasser B, Zimmer A, Khinast J, Roblegg E (2013) J Pharm Sci 102(11): 2987-3997.
6. Schrank S, Kann B, Saurugger E, Hainschitz M, Windbergs M, Glasser B, Khinast J, Roblegg E (2015) Int. J Pharm 478: 779-787.

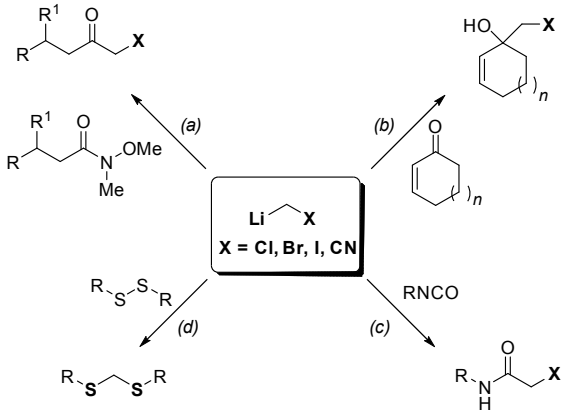
P-43

New Perspectives in Lithium Halocarbenoids Mediated Homologations in Medicinal Chemistry

Serena Monticelli, Azzurra Pelosi, Laura Castoldi, Giovanna Parisi, Marta Rui, Daniele Antermite, Ernst Urban, Thierry Langer and Vittorio Pace

Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, 1090 Vienna (Austria).

Organometallic compounds presenting a metal and at least one electronegative group (*e.g.* halogen, cyano) at the same carbon are referred as carbenoids.[1] Their chemistry is characterized by the intrinsic ambiphilicity between nucleophilic and electrophilic behaviour, depending – *inter alia* – on the nature of the metal.[2]
Ongoing research in our group deals with the employment of nucleophilic lithium halocarbenoids in homologation processes: effectively, the rapid and effective introduction of an halomethylenic fragment into a given electrophile would guarantee further synthetic elaborations. However, despite the synthetic usefulness of these reagents, thoroughly applications in organic synthesis have been somewhat limited by their inherent thermal instability. The reactivity of carbenoids towards electrophiles such as Weinreb amides,[3] heterocumulenes (*e.g.* isocyanates),[4] unsaturated carbonyls[5] and disulfides[6] will be presented (Scheme 1) jointly with mechanistic rationale and discussion on the stability of the intermediates involved in the transformations.



Scheme 1

1. Boche, G.; Lohrenz, J. C. W. *Chem. Rev.* 2001, *101*, 697-756; b) Pace, V. *Aust. J. Chem.* 2014, *67*, 311-313; c) Pace, V.; Luisi, R. *ChemCatChem* 2014, *6*, 1516-1519.
2. Capriati, V.; Florio, S. *Chem. Eur. J.* 2010, *16*, 4152-4162.
3. Pace, V.; Castoldi, L.; Holzer, W. *J. Org. Chem.* 2013, *78*, 7764-7770; b) Pace, V.; Holzer, W.; Verniest, G.; Alcántara, A. R.; De Kimpe, N. *Adv. Synth. Catal.* 2013, *355*, 919-926; c) Pace, V.; Holzer, W.; Olofsson, B. *Adv. Synth. Catal.* 2014, *356*, 3697-3736; d) Pace, V.; Castoldi, L.; Alcantara, A. R.; Holzer, W. *RSC Adv.* 2013, *3*, 10158-10162; e) Pace, V.; Castoldi, L.; Pregnolato, M. *Mini-Rev. Med. Chem.* 2013, *13*, 988-996.
4. Pace, V.; Castoldi, L.; Holzer, W. *Chem. Commun.* 2013, *49*, 8383-8385; b) Pace, V.; Castoldi, L.; Mamuye, A. D.; Holzer, W. *Synthesis* 2014, *46*, 2897-2909.
5. Pace, V.; Castoldi, L.; Holzer, W. *Adv. Synth. Catal.* 2014, *356*, 1761-1766.
6. Pace, V.; Pelosi, A.; Rosati, O.; Curini, M.; Holzer, W. *manuscript in preparation*.

We gratefully thank our sponsors for support