DPhG

Deutsche
Pharmazeutische
Gesellschaft e.V.

Annual Meeting of the German
Pharmaceutical Society – DPhG

Pharmaceutical Science: Structure, Function and Application

Hamburg, Germany
October 02 – 05, 2018
at Hamburg University

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

The Pharmaceutical Society of Japan

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Conference Book

Pharmaceutical Science: Structure, Function and Application

Annual Meeting of the German Pharmaceutical Society 2018 - DPhG

Hamburg, Germany, Oktober 2018

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www.2018.dphg.de

Institutional sponsors

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WELCOME ADDRESS

Dear colleagues and friends,

the President of the German Pharmaceutical Society (DPhG), Prof. Dr. Stefan Laufer, and the Congress Chairman of this year's annual meeting, Prof. Dr. Wolfgang Maison, would like to welcome you to Hamburg and Hamburg University. The focus of this year's meeting is „Pharmaceutical Science: Structure, Function and Application". A broad range of sessions for oral and poster presentations is going to cover important aspects of current pharmaceutical research.

We are looking forward to exciting talks, presented by many high level speakers and stimulating discussions. A particularly important part of the meeting is the interaction of younger scientists with other attendees. A dedicated session for poster short-talks will allow selected PhD students to present their work. Furthermore, two dedicated sessions for young investigators in the pharmaceutical field have been scheduled, offering an excellent platform for postdocs and young group leaders who have not yet received a professor position and need our full attention to secure growth and sustainability of academic pharmaceutical science in the future.

Many thanks to all who contributed to the assembly of this year's meeting, particularly the local helpers, the organizing committee, the scientific committee and of course you, the participants, for your contribution.

We are glad to have you here for a stimulating meeting in beautiful Hamburg and hope you will enjoy the scientific program as well as the location,

Prof. Dr. Stefan Laufer, President

Prof. Dr. Wolfgang Maison, Chairman

GENERAL INFORMATION

The Annual DPhG Meeting 2018 takes place at the Main Building of the Hamburg University (Edmund-Siemers-Allee 1).

LANGUAGE

The Conference language is English, no simultaneous translation will be provided.

INSTRUCTIONS FOR USING CONFERENCE WLAN

If your institution is member of the "eduroam" community, you can use the wireless network "eduroam". The configuration of your device should be the same as instructed by your home institution. Please use your account and the domain of your home institution.
If your institution is not member of the "eduroam" community, you can obtain a guest account and a password at the Conference office.

CONFERENCE OFFICE

The Conference office is located at the Conference building Edmund-Siemers-Allee 1, 20146 Hamburg.
Opening hours:
Wednesday, October 3rd, 2018: 10:00 – 18:00; in the foyer of the main building
Thursday, October 4th, 2018: 8:00 – 17:00; in the foyer of the main building
Friday, October 5th, 2018: 8:00 – 12:00; in the foyer of the main building

LIABILITY

The Organizers of the Conference cannot be held responsible for any loss, theft, damage or injury to any person or property during the Conference, whatever the cause may be. The liability of persons and enterprises providing means of transportations or other services remains unaffected. Each congress participant and accompanying person takes part in all tours at his/her own risk.

ABSTRACT AND POSTER NUMBERS

Each abstract has a unique identifier, a letter-number combination. Letters refer to the conference topic a contribution was assigned to (i.e. plenary lectures are identified by the letter "P", scientific lectures by the letters "SL", and poster presentations by the letters "POS"). Please note that in case of poster presentations the abstract number is identical with the poster number.
Please refer to the authors index on page 177 for direct access to specific abstracts.

POSTER SESSIONS

Posters with the following topics are located at the west wing: Other Topics, Medicinal chemistry and drug design, Antiinfectives, Cancer, Inflammation.
Posters with the following topics are located at the east wing: Analytics, Biotechnology and Biopharmaceutics, Clinical Pharmacy, Natural Compounds, Pharmaceutical Technology and Biomaterials, Pharmacology.
Presenting authors are asked to be present at their poster during the poster session.

CONFERENCE DINNER

Separate registration necessary (special fee). Please refer to the Conference office for registration and details. The Conference dinner will take place at "Hotel Hafen Hamburg", Seewartenstraße 9, 20459 Hamburg.

BADGES

Badges will be issued to all registered participants and enable access to all scientific sessions.

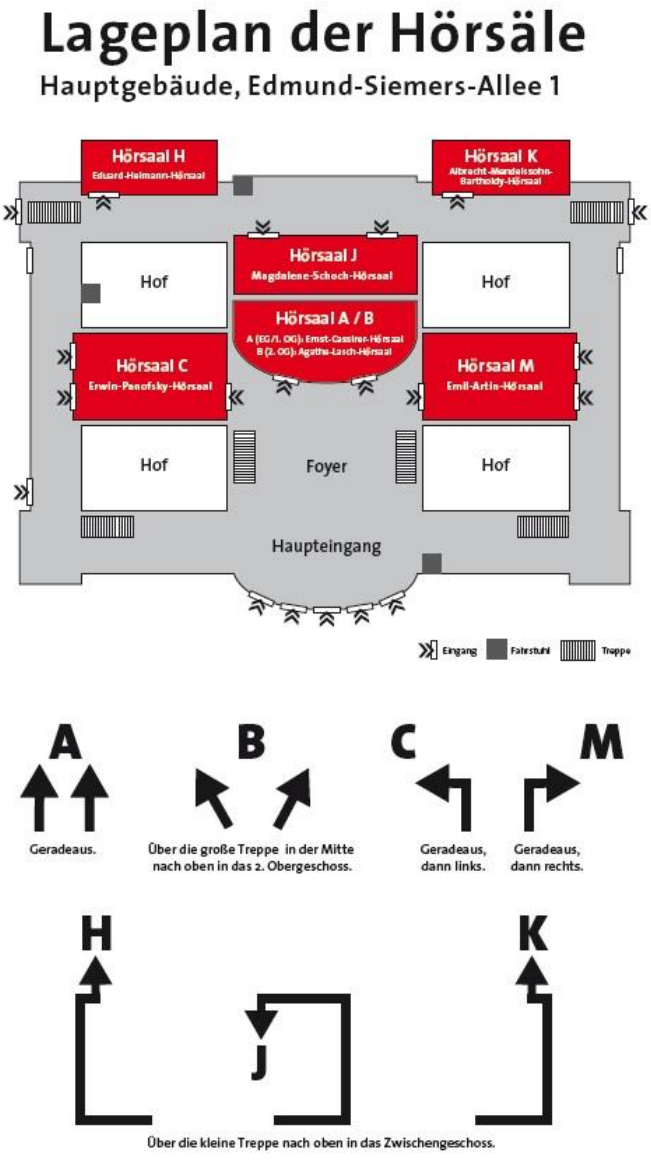
LOCATIONS

The Congress will take place at Hamburg University's Main Building, Edmund-Siemers-Allee 1, 20146 Hamburg, opposite to Dammtor train station.

It's easy to get to Hamburg University's Main Building by public transportation via S-Bahn-trains or bus.

The S21 and S31 trains take three minutes to get from the Main Station (Hauptbahnhof) to Dammtor Train Station (main campus). The 4, 5 and 109 buses go directly to the University (Dammtor (Messe/CCH) bus stop).

Due to limited parking, we strongly recommend taking public transportation!



Hotel Hafen Hamburg (Conference Dinner):

The Hotel Hafen Hamburg is located right by the Hamburg harbour, approx. 2.5 km from the Hamburg University.

BY PUBLIC TRANSPORTATION

Go from station Hamburg Dammtor (3 mins walk) to Hamburg main station by S21 or S31. Take the S-Bahn S1 or S3 to Hamburg Landesbrücken (5 mins walk to destination).

Go from station Hamburg Dammtor (3 mins walk) to Rathausmarkt by bus line 5. Have a 3 mins walk to station Rathaus and take Underground line 3 to station Landungsbrücken (3 mins walk to destination).

Stations:

- S-Bahn: S1, S3 Landungsbrücken/St. Pauli
- U-Bahn: U3 Landungsbrücken/St. Pauli
- Bus: 112 St.Pauli Hafenstraße/Landungsbrücken

CONFERENCE PROGRAM OVERVIEW

Pre-Meeting Program

Tuesday, October 2nd	
Vorsymposium der Fachgruppe „Geschichte der Pharmazie“: *Pharmazie in Hamburg* **Ort: Hamburg University Main Building, Edmund-Siemens-Allee 1, 20146 Hamburg, HS A**	
14:00 – 14:15	Begrüßung durch den Vorsitzenden der FG Geschichte der Pharmazie *Prof. Dr. Christoph Friedrich, Marburg*
14:15 – 15:00	Zur Entwicklung des Hochschulfaches Pharmazie an der Universität Hamburg *Marie-Krystin Borchers, Reinbek*
15:00 – 15:45	Zur Entwicklung des Apothekenwesens in Hamburg *Dr. Frederick Vongehr, Moers*
15:45 – 16:15	Kaffeepause
16:15 – 17:00	Zur Geschichte der Hamburger Firma Beiersdorf *Dr. Stefanie Boman-Degen, Osnabrück*
17:00 – 17:45	Das Bernhard-Nocht-Institut für Tropenmedizin in Hamburg und sein Einfluss auf die Arzneimittelentwicklung in der ersten Hälfte des 20. Jahrhunderts *Dr. Ute Jutta Götz, Herschbach*

Wednesday, October 3rd Main Symposium (Congress language English)				
09:00 – 12:00	**Sitzung des VdPPHI (HS B)**			
13:00 – 13:45	**Opening of the Annual DPhG Meeting 2018 (HS A)** **Pharmaceutical Science: Structure, Function and Application**			
13:45 – 14:30	**Plenary Lecture 1, L. Carrier,** *Applications and perspectives of CRISPR/Cas9* **(HS A)** **P.1**			
14:30 – 15:00	**Coffee Break**			

SHORT TALKS (parallel sessions I)

15:00 – 16:30	SL1 (HS M)	SL2 (HS B)	SL3 (HS A)	SL4 (HS J)
	Cannabis and Cannabinoids: Quo vadis? Chairs: B. Hinz, T. Efferth	**Computational Approaches in Pharmaceutical Research** Chairs: J. Kirchmair, G. Wolber	**Laboratory Automation** Chair: H. Wätzig	**cGMP-Signalling** Chairs: J. Schloßmann, P. Ruth
	15:00 **SL.01** **B. Hinz**: *Cannabinoids for Medical Use: A Pharmacologist´s View*	15:00 **SL.04** **D. Schmidt**: *Structure-based workflow to deorphanize cryptic allosteric sites*	15:00 **SL.08** **R. Wiesner:** *Important features of lab automation: an overview*	15:00 **SL.11** **R. Lukowski**: *Cardioprotection by cGMP and its signal transduction to mitochondria*
	15:30 **SL.02** **R. Thomasius:** *Cannabis use from the perspective of addiction medicine – with special consideration of adolescence*	15:30 **SL.05** **M. Rarey:** *For Occasional Use: Cheminformatics and Structure-Based Design Tools for Medicinal Chemists*	15:30 **SL.09** **M. Stephan:** *Lab automation; state of the art and future prospects*	15:30 **SL.12** **V. O. Nikolaev:** *Physiological effects of cGMP in heart muscle cells – a matter of compartmentation*
	16:00 **SL.03** **H. Reimann**: *Pharmaceutical aspects of cannabis and cannabinoids for medicinal use*	16:00 **SL.06** **M. Bermudez**: *In Silico Pharmacology – Mechanistic Receptor Models for Rational Drug Design*	16:00 **SL.10** **P. Zucchelli:** *Robotics as a tool for the 21st century biology*	16:00 **SL.13** **A. Schmidtko**: *cGMP-dependent signaling pathways in chronic pain processing*
		16:15 **SL.07** **D. Merk:** *Artificial intelligence driven de novo molecular design for nuclear receptor ligand discovery*		
16:45 - 17:30	**Plenary Lecture 2, B. Meibohm,** *The Role of Pharmacokinetic/Pharmacodynamic Evaluations in the Preclinical Development of Spectinamides, a Novel Class of Anti-Tuberculosis Agents* **(HS A)** **P.2**			
	Meetings der DPhG-Fachgruppen			

17:30 – 18:45	**Fachgruppe Pharm./Med. Chemie** P. Gmeiner (HS A)	**Fachgruppe Pharm. Biologie** A. Vollmar (HS H)	**Fachgruppe Pharma-kologie** J. Klein (HS J)	**Fachgruppe Pharm. Technologie** D. Fischer (HS M)	**Fachgruppe Klinische Pharmazie** T. Lehr (HS K)
19:00 – 21:30	**Poster Session and Welcome Reception**				

Thursday, October 4th

SHORT TALKS (parallel sessions II)

08:30 – 10:00	SL5 (HS A)	SL6 (HS B)
	New Research, New Researchers I Chair: S. Laufer	**3D(ifferent) Views of Antibiotic Resistance** Chairs: P. Heisig, A. Heisig
	08:30 **SL.14** **D. Lunter:** *Confocal Raman microspectroscopy as an alternative method to characterize stratum corneum lipid content and conformation*	**08:30** **SL.19** **D. Wilson:** *David versus Goliath: Ribosome-target antibiotics and bacterial resistance mechanisms*
	08:45 **SL.15** **T. Lühmann**: *Nanomechanics of fibroblast growth factor 2 – heparin at physiological and pathophysiological conditions: Implications for drug delivery*	
	09:00 **SL.16** **M. Koziolek**: *The rapid onset of plasma concentrations after oral administration of a novel Aspirin® formulation in the fed state can be explained by the presence of the Magenstraße*	**09:00** **SL.20** **P. Heisig:** *3D(ifferent) Views of Antibiotic Resistance – the Cellular View: Bacterial Strategies to Develop High-Level Fluoroquinolone Resistance*
	09:15 **SL.17** **C. Wischke**: *A multimaterial system for on-demand protein release*	
	09:30 **SL.18** **M. Dwivedi**: *Polysorbate degradation in biotherapeutic formulations: Identification and discussion of current root causes*	**09:30** **SL.21** **S. Günther:** *3D(ifferent): Views of Antibiotic Resistance: The environmental view*
10:00 – 10:15	**Coffee Break**	

SHORT TALKS (parallel sessions III)

10:15 – 11:15	SL7 (HS A)	SL8 (HS B)
	New Research, New Researchers II Chair: A. Link	**Pharmacometrics for *In Vitro* Models, Mice, and Men** Chairs: C. Kloft, S. Wicha
	10:15 **SL.22** **K. Bartel:** *Connecting lysosomes and mitochondria in cancer - regulation of lipid metabolism and mitochondrial function by the V-ATPase*	**10:15** **SL.26** **J. Borghardt:** *Human PK prediction - a generic approach combining both in vitro and in vivo preclinical data*

	10:30 **SL.23** **S. Moser:** *Phyllobilins – An Underexplored Family of Natural Products that have an Effect on Cancer Cells*	**10:35** **SL.27** **T. Lehr:** *Translational PK/PD modelling of ISTH0036, a potent and selective antisense oligonucleotide targeting transforming growth factor beta 2 (TGF-β2) for the treatment of ophthalmic diseases*
	10:45 **SL.24** **A. Brunschweiger:** *Avoiding depurination in the synthesis of DNA-tagged tetrahydroquinolines by micellar Brønsted acid catalysis*	**10:55** **SL.28** **S. Völler:** *Pharmacokinetic and pharmacodynamic studies aiming for rational drug dosing in preterm neonates: the DINO study*
	11:00 **SL.25** **S. Lüdeke:** *The thermodynamics of elastin-like protein (ELP) assembly: a circular dichroism-based approach*	
	Poster Short Talks	

11:15 – 12:15	**Poster Short Talks (HS A)** Chair: A. Link	
	11:15 **PST.01** **T. Drazic:** *Peptidomimetic β-lactams as electrophilic warheads in flaviviral protease inhibitors*	**11:40** **PST.06** **R. Schwenk:** *Investigations into the influence of pretubulysin (PT) and other microtubule-targeting agents (MTAs) on leukocyte-endothelial cell interactions*
	11:20 **PST.02** **M. Meßner:** *Switching lanes - Metabolic implication of Tigecycline as an efficacious second-line treatment for patients with sorafenib-resistant Hepatocellular Carcinoma*	**11:45** **PST.07** **F. K. Mwiiri:** *Electrospun Bioactive Wound Dressing containing Nanodispersions of Birch Bark Extract*
	11:25 **PST.03** **R. Hofstetter:** *Fully validated supercritical-fluid chromatography (SFC) for simultaneous quantification of acidic and basic metabolites of the divisive K^+ channel opener flupirtine*	**11:50** **PST.08** **J. Krieghoff:** *Three-armed biodegradable material platform providing adjustable degradation properties*
	11:30 **PST.04** **S. Franck:** *An in vivo-in silico approach to assess the pharmacokinetics of a novel concept combining amoxicillin with an immunomodulatory drug.*	**11:55** **PST.09** **M. Morsi:** *Effect of cell culture on the biphasic kinetics of insulin secretion*
	11:35 **PST.05** **S. Zimmermann:** *Minimally invasive, model informed drug monitoring of tyrosine kinase inhibitors*	**12:00** **PST.10** **A. Aigner:** *The inhibition of c-met in gastric cancer cells leads to the upregulation of HER3 via SATB1*
12:15 – 13:15	**Lunch break**	
13:15 – 14:00	**Plenary Lecture 3, L. Meinel,** *Bioinspired Drug Delivery Systems and Diagnostics* **(HS A)** **P.3**	

SHORT TALKS (parallel sessions IV)

	SL9 (HS B)	SL10 (HS A)	SL11 (HS J)
14:15 – 15:45	**Gene Therapy** Chairs: E. Oetjen, L. Carrier	**Biomimetic and Stimuli-Responsive Biomaterials** Chairs: D. Fischer, G. Fuhrmann	**Mass Spectrometry** Chair: M. Lämmerhofer
	14:15 **SL.29** **M. Biel:** *Gene supplementation therapy of CNGA3-linked achromatopsia*	**14:15** **SL.32** **I. Herrmann:** *Nanoparticle-based Tissue Adhesives with Biomimetic Activity*	**14:15** **SL.38** **H. Schlüter:** *Proteome analysis by mass spectrometry*
	14:45 **SL.30** **M. Trepel:** *Gene Therapy - limitations of viral gene transfer and possible solutions*	**14:35** **SL.33** **A. Lendlein:** *Nano Carriers and Structured Hydrogels containing Amino Acids*	**14:45** **SL.39** **K. Wanner:** *MS Binding Assays for the Characterization of Ligand Affinity and Drug Screening*
	15:15 **SL.31** **M. Schwaninger:** *Endothelium as a target for the gene therapy of brain diseases*	**14:55** **SL.34** **G. Fuhrmann:** *Extracellular vesicles as biogenic carriers for therapeutic applications*	**15:15** **SL.40** **N. Ferreirós Bouzas:** *Pitfalls of lipid mediator quantification in biological matrices using LC-MS/MS*
		15:10 **SL.35** **D. Klinger:** *Amphiphilic Nanogels: Tailoring Network Hydrophobicity to Dermal Delivery Applications*	
		15:25 **SL.36** **S. Braig:** *Antitumoral potential of the myxobacterial peptolide Vioprolide*	
		15:35 **SL.37** **K. Mäder:** *Stimulus sensitive HPMA polymers for tumour therapy: stimulus selection, optimisation and in vivo performance*	
15:45 – 16:15	**Poster Session Poster Short Talks and Coffee Break**		
16:15 – 17:00	**Plenary Lecture 4, H. Abken,** *CAR T cells on the highway to clinical application* **(HS A)** **P.4**		
17:30 – 19:00	**DPhG Hauptversammlung (HS A)**		
From 19:30	**Conference Dinner (Hotel Hafen Hamburg, Seewartenstraße 9, 20459 Hamburg)**		

Friday, October 5th

SHORT TALKS (parallel sessions V)

08:30 – 10:00	SL12 (HS A)	SL13 (HS B)	SL14 (HS J)
	Antibacterial Natural Products Chairs: C. Ducho, R. Holl	**From Structure to Function with Modern Techniques in Structural Biology** Chair: C. Betzel	**Clinical Pharmacy to Improve Medication Safety** Chairs: C. Langebrake, M. J. Hug
	08:30 SL.41 **C. Ducho:** *Nucleoside Antibiotics as Antibacterial Agents*	08:30 SL.45 **A. Mancuso:** *Serial crystallography at the European XFEL: First results and future opportunities*	08:30 SL.48 **M. Schultze-Florey:** *Population pharmacokinetics of midazolam in combination with fentanyl in critically ill children*
	08:55 SL.42 **M. Brönstrup:** *Induction and quantification of drug uptake into Gram-negative bacteria*	08:50 SL.46 **D. Oberthuer:** *Serial Crystallography - Exciting possibilities for time resolved Structural Biology*	08:50 SL.49 **M. Baehr:** *Closed Loop Medication Administration – Medication Safety for Inpatients*
	09:20 SL.43 **H-D. Arndt:** *Synthesis and Chemical Biology Studies on Thiopeptide Antibiotics Structure and Activity*	09:10 SL.47 **S. Glöckner:** *Thermodynamic and Kinetic Elucidation of Protein-Ligand Interactions by Kinetic Isothermal Titration Calorimetry and Macromolecular Crystallography*	09:10 SL.50 **G. Först:** *The Role of Pharmacists in Antibiotic Stewardship Teams*
	09:45 SL.44 **N. Schützenmeister:** *The Hunt for Novel Antiinfective Compounds - Synthesis of Rubrolide Analogues*		09:30 SL.51 **A. Förster:** *"AMTS, ARMIN, ATHINA": Clinical pharmacy in the ambulatory care setting of Germany – an update on the current path*
10:00 – 10:30	**Coffee Break**		

SHORT TALKS (parallel sessions VI)

10:30 – 12:00	SL15 (HS J)	SL16 (HS A)	SL17 (HS B)	SL18 (HS C)
	Epigenetics Chairs: M. Jung, F. Hansen	**Protein Kinase Inhibitors - Novel Inhibition Mechanisms** Chairs: S. Laufer, C. Kunick	**Drug Delivery of Poorly Water Soluble Molecules - from Salt Design to Carriers** Chairs: L. Meinel, H. Bunjes	**Supercritical Fluid Chromatography** Chair: M. Parr

	10:30 **SL.52** **U. Oppermann**: *Chemical Tools to advance epigenetic biology and drug discovery*	**10:30** **SL.57** **C. Kunick**: *Halogen bonding as concept for protein kinase inhibitor design*	**10:30** **SL.62** **K. Wagner:** *From Molecule to Molecular Dispersion – Enabling Formulations via Hot-Melt Extrusion*	**10:30** **SL.65** **T. Berger**: *Beyond the Current State-of-the-Art in Pharmaceutical Analysis using Supercritical Fluid Chromatography (SFC)*
	11:00 **SL.53** **S. Günther:** *Target discovery beyond the BET-Family: some novel chemical probes for less studied bromodomains*	**10:50** **SL.58** **S. Laufer**: *Targeting the R-Spine: Design, Synthesis and Biological Evaluation of Novel Type I½ p38α MAP Kinase Inhibitors with Excellent Selectivity, High Potency and Prolonged Target Residence Time: Implication for Cancer- and CNS-Applications*	**11:00** **SL.63** **K. Mäder:** *Rational design and characterization of lipid based DDS for oral administration of poorly soluble drugs.*	**11:00** **SL.66** **S. Bühler:** *Application of SFC(MS) in Drug Research and Development*
	11:20 **SL.54** **D. Robaa:** *Structure-based design of selective HDAC8 inhibitors*	**11:10** **SL.59** **K. Huber:** *Investigating Cellular Mechanism-of-Action and Target Engagement with Chemical Biology*	**11:30** **SL.64** **J. Wiest:** *Steering the interplay of supramolecular species (aggregates, micelles, vesicles) in the gastrointestinal tract – a new paradigm in enabling formulations development*	**11:30** **SL.67** **J. Joseph**: *SFC-MS in bioanalysis*
	11:35 **SL.55** **F. Hansen:** *Development of RTS-V5 as the first-in-class dual histone deacetylase-proteasome inhibitor*	**11:30** **SL.60** **P. Koch**: *Reversible and irreversible inhibitors of c-Jun N-terminal kinase 3*		
	11:50 **SL.56** **R. Simon:** *Cofactor-based Chemical Probes for Lysine Acetyltransferases*	**11:45** **SL.61** **M. Ardelt**: *Blocking Traffic Flow – Cdk5 Inhibition Introduces Novel Ways to Prevent Sorafenib Treatment Escape in HCC Cells*		

Lunch Break

Plenary Lecture 5, G. Klebe, *Small, Weak but Beautiful: Why are Fragments Promising Starting Points in Drug Development* **(HS A)** **P.5**

Closing Ceremony (HS A)

Post-Meeting Program

Saturday, October 6th	
15:00 – 18:30	**Tag der Offizinpharmazie** in Kooperation mit der Apothekerkammer Hamburg und FG Allgemeinpharmazie der DPhG Ort: Universität Hamburg, Institut für Pharmazie, Hörsaal Technische und Makromolekulare Chemie (TMC), Bundesstraße 45, 20126 Hamburg
	Bessere Therapien durch AMTS? Was Apotheker zur Therapieoptimierung beitragen können
15:15	**Prof. Dr. Rolf Daniels, Lehrstuhl für Pharmazeutische Technologie, Universität Tübingen** Inhalativa – was bei einer Substitution zu beachten ist
15:45	**Dr. Juliane Kresser, Fürth, Produktmanagerin der Lauer Fischer AG Fachbereich Medikationsmanagement, AMTS** AMTS im Zeitalter der Digitalisierung
16:15	**Pause**
16:46	**Dr. Olaf Rose, Pharm.D., Elefanten-Apotheke, Steinfurt** Medikationsanalyse in der Praxis
17:30	**Panel mit den Referenten und Publikumsbeteiligung**

ANMELDUNG

Eine Anmeldung zum Tag der Offizinpharmazie ist nicht erforderlich.

Die Teilnahme ist kostenlos.

Die Veranstaltung wird mit 4 Punkten zertifiziert.

www.dphg.de/apo18

1 PLENARY LECTURES

P.1

Applications and perspectives of CRISPR/Cas9

L. Carrier

Institute of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany

Initially found in bacteria as an adaptive immune response, CRISPR/Cas9 has been shown to also act as a designer nuclease with specific engineered single guide RNA. Therefore, CRISPR/Cas9 revolutionized the field of genome editing. My lecture will be a comprehensive overview of genome editing with CRISPR/Cas9 and related systems for different applications, including the development of human and animal models of disease and therapies. I will also share different strategies of gene therapy in the treatment of hypertrophic cardiomyopathy, which is one of the most prevalent inherited disorders. Many examples will be given in particular in the field of cardiovascular research and medicine.

P.2

The Role of Pharmacokinetic/Pharmacodynamic Evaluations in the Preclinical Development of Spectinamides, a Novel Class of Anti-Tuberculosis Agents

Bernd Meibohm, PhD, FCP, FAAPS

Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, Tennessee, USA

Although the classical antibiotic spectinomycin is a potent bacterial protein synthesis inhibitor, poor antimycobacterial activity limits its clinical application for treating tuberculosis. Using structure-based design, we generated a new semisynthetic series of spectinomycin analogs with selective ribosomal inhibition and excellent narrow-spectrum antitubercular activity. The derived spectinamides are a novel class of antitubercular agents with the potential to treat drug-resistant tuberculosis infections. Their antitubercular activity is derived from both ribosomal affinity and their ability to overcome intrinsic efflux mediated by the Mycobacterium tuberculosis Rv1258c efflux pump. Spectinamides follow a narrow structure-activity relationship, consistent with a tight ribosome-binding pocket and strict structural requirements to overcome native bacterial efflux. In multiple murine infection models, spectinamides were well tolerated, significantly reduced lung mycobacterial burden and increased survival. *In vitro* studies demonstrated a lack of cross resistance with existing tuberculosis therapeutics, and activity against multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. This desirable antimicrobial spectrum was matched by an excellent pharmacological profile including low protein binding, low microsomal metabolism, no cytotoxicity, and high tissue penetration. Recent *in vivo* combinations studies suggest that spectinamides are effective partner agents for multiple established anti-tuberculosis agents including bedaquiline, rifampicin and pyrazinamide.

While pharmacometric analyses have in the past largely been focused on clinical data, more recent efforts are focused on a more widespread application also in preclinical drug development. The presentation will focus on the rigorous application of PK/PD characterizations and modeling and simulation approaches to prioritize candidate compounds in the drug discovery and early development space. With spectinamides as example, the presentation will illustrate how pharmacometric approaches have been applied to facilitate lead candidate selection in *in vitro* and *in vivo* experiments, and to characterize the dose-exposure-response relationship of lead spectinamides to facilitate the selection of optimal dosing regimens and drug combination partners in further development. It will furthermore show how pharmacometric assessments provided a rationale for selecting intrapulmonary aerosol delivery as an alternative treatment route for spectinamide anti-tuberculosis agents.

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P.3

Bioinspired Drug Delivery Systems and Diagnostics

Meinel L.[1]

[1] *Institute of Pharmacy and Food Chemistry, University of Würzburg, 97074, Germany*

The featured bioresponsive diagnostics and therapeutics operate in a 'fire and forget' mode in that these autonomously respond to (patho-)physiological changes. Activation is by proteolytic digest of prodrugs or "pro-diagnostics", effectively resulting in drug activation or signal generation for therapeutic and diagnostic use, respectively. Two developments are exemplarily highlighted, one featuring Interleukin-4 (IL-4) for tissue painting and bioresponsiven activation in joint cavities (application osteoarthritis) and another one featuring a diagnostic chewing gum, responding to inflammation within the oral cavity (application periodontitis).

P.4

CAR T cells on the highway to clinical application

Hinrich Abken
Regensburg Center for Interventional Immunology (RCI), Chair Gene-Immunotherapy,
University Hospital Regensburg, Regensburg, Germany
hinrich.abken@ukr.de

Adoptive therapy with chimeric antigen receptor (CAR) modified T cells achieved spectacular remissions of so far refractory leukemia/lymphoma, mostly in the treatment of B cell malignancies, resulting in lasting remissions. Several strategies are currently explored to prevent relapse and to achieve a long-term control of the disease. The treatment of solid tumors also showed some efficacy, the successful treatment of solid cancer still remains challenging. This is thought to be due to the hostile conditions of the tumor stroma and the repressive immune cell infiltrate. In new developments CAR T cells are being used as redirected “living factories” to deposit immune modulating cytokines in the tumor tissue aiming at converting the immune cell environment into a more favorite one to sustain a productive anti-tumor response. IL-18 releasing CAR or TCR modified T cells showed superior anti-tumor activities in several tumor models. Such IL-18 TRUCKs or “fourth generation” CAR T cells are going to change our concepts of treating tumors and delivering drugs to pre-defined lesions in the near future.

P.5

Small, Weak but Beautiful: Why are Fragments Promising Starting Points in Drug Development

Klebe, G.

Institute of Pharmaceutical Chemistry, Philipps-Universität Marburg, Marbacher Weg 6, 35037, Marburg, Germany

Fragment-based lead discovery has become increasingly popular to identify first leads for drug development. Advantage of using fragments is their low molecular weight, which leaves sufficient space for subsequent chemical optimization before the pharmaceutically reasonable limit of 500 g/mol is reached. However, the inherent low-binding affinity of fragments poses major challenge to current biophysical and biochemical screening methods and questions whether such techniques can be used as pre-screening filter to reliably detect all putative fragments subsequently to be crystallized with the target protein. Since data collection at synchrotrons has become fast enough, to consider direct crystallographic fragment screening on protein crystals. We designed a 1270-entry fragment library and tested a 96 compound subset against seven different proteins with a mean hit rate of 17%. Against the aspartic protease endothiapepsin, we selected a 361-entries and tested this collection with six biophysical methods such as STD-NMR, ESI-MS, thermal shift analysis, micro-thermophoresis, high-concentration enzyme and reporter displacement assay and crystallography to identify putative binders.[1] Each individual screening method revealed varying hit rates with only a minimal overlap of commonly shared hits. Remarkably, the direct screening on protein crystals resulted in 71 crystal structure, but, disappointingly, nearly half of the hits were missed by any of the biophysical screening methods.[2] We therefore strongly advocate screening fragments directly on protein crystals instead of applying elaborated pre-filters, as the determination of fragment-bound crystal structures is pivotal for any subsequent design project to evolve a fragment into a drug candidate. However, how to optimize weak initial fragment binders to putative drug leads? We developed several design tools to search for analogs, finding larger ligands embedding to original core fragment or allowing a tailored docking of putative side chains to the initial core fragments. Furthermore, since natural product-like compounds are claimed to be better starting points for drug development, we used the AnalytiCon FRGx fragment collection to discover initial hits by crystallography.[3] Subsequently, we identified by docking putative lead candidates that embed the core fragment into a larger scaffold available in the 41,000 entry NATx database of natural compounds. Fragments are ideal probes to map the properties of binding sites, apart from suggesting promising seeds to embark onto drug discovery projects.[4,5] Binding of fragments is strongly influenced by the presence and interaction with water molecules.[6] Such contacts select, mediate, and stabilize the actually adopted fragment poses. Even though fragments are very small and the putative chemical space to be considered is significantly reduced, their space mapping molecular recognition principles is extremely large, nevertheless, fragments seem to be bind highly specific and selective. Adopted geometries are strongly modulated by the local pKa conditions. Neutron diffraction even provided us important insights into protonation states and local hydrogen-bonding patterns around bound fragments.[7]

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2 SCIENTIFIC LECTURES

2.1 Cannabis and Cannabinoids: Quo vadis?

Chairs: B. Hinz, T. Efferth

SL.01

Cannabinoids for Medical Use: A Pharmacologist´s View

Hinz B

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The medical knowledge of Cannabis and its ingredients is still limited. An essential impetus for a consequent preclinical and clinical research in this field was even given with the identification of the endocannabinoid system by the beginning of the 1990s. As a matter of fact, the findings obtained ever since have been contributed to an increased acceptance of cannabinoids as therapy option within past years. However, in addition to the currently available clinical data, further large randomized clinical studies are absolutely necessary to substantiate and confirm the efficacy of cannabinoids in specific indications. This talk will address the molecular mechanisms underlying pharmacological effects of cannabinoids as well as the current evidence-based knowledge of their medical use. Moreover, an outlook on forthcoming trends in this area will be provided.

SL.02

Cannabis use from the perspective of addiction medicine – with special consideration of adolescence

R. Thomasius

Current research results show that pubertal and adolescent cannabis use can result in serious somatic and mental diseases, in continuing disruption of age-specific developmental and growth processes, in academic failure as well as in the social disintegration of those concerned. The risk of rapidly developing an addiction from cannabis, including the use of hard illegal drugs, is especially high in children and adolescents. Today, cannabis use is the primary reason for patients to seek addiction counselling and treatment for the first time when illegal drugs are concerned. The success rate for treatment is low. The best protection for children and adolescents at risk for addiction is to combine adequate steps for reducing demand and reducing supply of available drugs. Legalizing cannabis products therefore is a step in the wrong direction. It would jeopardize the former route taken by drug politics, which has been a success in comparison to European neighbours, and would hit hard on specifically those children and adolescents, who grow up in disadvantaged social conditions.

SL.03

Pharmaceutical aspects of cannabis and cannabinoids for medicinal use

H. Reimann

Deutscher Arzneimittel-Codex/Neues Rezeptur-Formularium (DAC/NRF)

Avoxa - Mediengruppe Deutscher Apotheker GmbH
Apothekerhaus Eschborn

Cannabis flos DAB (dried flowertops of the whole female flower), several standardised cannabis extracts, cannabidiol DAC (CBD), and dronabinol DAC (THC) are available as the starting material for pharmacy preparation. The unlicensed medicinal products are individually prepared extemporaneously for a specific patient in line with the practitioner's prescription. Since formula are given in the NRF part of Deutscher Arzneimittel-Codex/Neues Rezeptur-Formularium (DAC/NRF) for different dosage forms for oral use and for inhalation, practitioners will typically follow recommended patterns when prescribing cannabis and cannabinoids for medicinal use. Clarification may be necessary if the prescription explicitly states the delivery of the whole flower or if instructions for the patient are incomplete. Presently there is no need to prescribe cannabis flos according to the precise CBD and THC content, but stating one of the strains referring to a specific range for the nominal values of the cannabinoids is sufficient. The NRF formula on the cut herbal drug for decoction or for vaporisation, on oily oral drops and on ethanol-based drops for vaporisation offer detailed and feasible instructions for preparation. These include single-use and multiple-use containers, childproof closures and appropriate administration devices as well as information on storage and in-use stability. The DAC monographs and the ancillary texts on the identification of the cannabinoids, the herbal drug and the extracts include macroscopic and microscopic examination, thin layer chromatography, and melting point. The tests are generally feasible, but the DAC/NRF commission step up effort to consider cost-saving and time-saving options, e. g. infrared absorption photospectrometry.

2.2 Computational Approaches in Pharmaceutical Research

Chairs: J. Kirchmair. G. Wolber

SL.04

Structure-based workflow to deorphanize cryptic allosteric sites

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Allosteric modulators have a huge potential to overcome typical hurdles in drug design. Here we present a workflow to deorphanize cryptic allosteric sites that consists of four steps.

I) Allosteric regulation may be possible via so-called “cryptic sites”. Cryptic sites are closed in their apo state and, hence, are inherently difficult to identify. Yet, they have been predicted to exist on many proteins. [1] We have developed an approach to sample, identify, and rank cryptic sites on protein structures using cosolvent-based molecular dynamics simulations. Using this approach, we were able to reproduce the open cryptic site conformation, including the docked ligand pose, starting from the closed conformation, for 5 of our 7 test systems.

II) However, not all cryptic sites might functionally be coupled to the orthosteric site of a protein. To probe for such a potential influence, we developed Constraint Network Analysis (CNA) as a framework to analyze the coupling between putative binding sites by rigidity theory. CNA has been successfully validated to be able to quantify allosteric coupling. [2] This approach was used to classify ligands of GPCRs with respect to their functional bias.

III) To now predict if an orphan cryptic site allosterically couples to the orthosteric one, we developed molecule-like de novo scaffolds (“Fuzzy Ligands”) to probe for a potential allosteric communication with CNA. [3]

IV) In case of a positive response, the Fuzzy Ligands then serve as queries for downstream virtual screening approaches.

Overall, our four-step workflow should allow targeting (yet unknown) allosteric sites in structure-based drug design, taking into account binding and functional properties of the ligand candidates.

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SL.05

For Occasional Use: Cheminformatics and Structure-Based Design Tools for Medicinal Chemists

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The use of cheminformatics and computational molecular design methods is common practice in pharmaceutical research. For several decades, the application of computational methods was reserved to computational chemistry experts. Although this is still the case for many complex computational tasks, with more and more data easily available in digital form, computational methods become part of the tool arsenal of every medicinal chemist. Since chemists are mostly occasional users, important software requirements are simple, intuitive interfaces and reliability. As a consequence, a high level of automatization is necessary making the development of new methods challenging. Over the past decade, we developed several software tools and web services aiming at a good trade-off between simplicity and productivity.[1-3] In this talk we present a few showcases from the field of cheminformatics and protein structure-based design.[4]

Acknowledgments: BioSolveIT GmbH, Christian Lemmen, Marcus Gastreich, and colleagues for a long-standing joint software development cooperation.

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SL.06

In Silico Pharmacology – Mechanistic Receptor Models for Rational Drug Design

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In this talk we will demonstrate the value of computational methods for the mechanistic understanding of complex functional roles of G protein coupled receptors (GPCRs). GPCRs represent integrative and highly dynamic signalling machines, transferring information across membranes via multiple signalling pathways. Although about 30 % of all marketed drugs directly target GPCRs, little is known about the ligand-induced conformational changes that lead to intracellular signalling. Most of the drugs targeting GPCRs were developed with the assumption that GPCRs are simple on-off-switches. Over the last decade, specific receptor-ligand complexes were determined by crystallography providing indispensable structural insights. This structural revolution fostered the elucidation of complex signalling functions of this protein class, including allosteric modulation, partial agonism or biased signalling (functional selectivity). However, these crystal structures represent single static conformations of highly flexible proteins. For a better understanding of receptor functionality we need mechanistic models that consider GPCRs as dynamic entities.

We will explain how computational approaches can be guided by pharmacological experiments and unveil mechanisms of specific receptor functions. A deep mechanistic understanding of GPCR functionality is essential for the rational design of tailor-made GPCR modulators. We will present some case studies focusing on partial receptor activation and biased signalling, which highlight the explanatory power of computationally-derived receptor models and their usage for rational drug design.

SL.07

Artificial intelligence driven de novo molecular design for nuclear receptor ligand discovery

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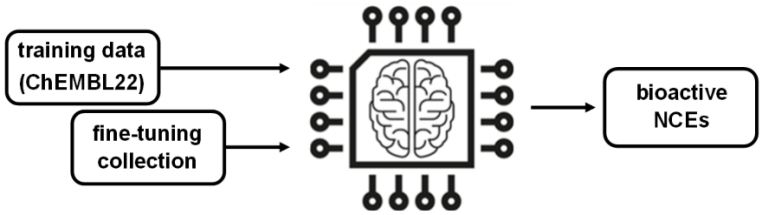
Instances of artificial intelligence (AI) coupled with the availability of large chemical and biological datasets enable data-driven drug discovery[1]. We have applied a generative machine learning strategy based on a deep recurrent neural network (RNN) for de novo molecular design[2]. The computational model was first trained to capture the grammar of SMILES representations of drug-like small molecules, and then used to automatically generate SMILES strings of new chemical entities (NCEs). Transfer learning on small collections of bioactive templates enables fine-tuning of the model to generate target-focused sets of molecules. In a pioneering prospective study[3], the generative RNN was trained on small molecules (540'000) from a public compound database (ChEMBL22) and fine-tuned on a set of 25 fatty acid mimetics with known activity on two nuclear receptors (RXR, PPAR). The computationally designed samples from this model resembled drug-like molecules and comprised favourable synthetic accessibility. Five top-ranked examples were selected for synthesis and biological characterization. Four designs were active on the studied nuclear receptors in specific hybrid reporter gene assays with up to nanomolar potencies (EC50 0.13 - 14 µM), resembling the activities of the fine-tuning set (EC50 0.024 - 31 µM). These results confirm the potential of AI-driven de novo design for the discovery of synthetically accessible and bioactive NCEs as lead compounds for medicinal chemistry.



After this successful proof-of-concept application[3], we studied the potential of generative AI models for the de novo design of bioactive natural product mimetics[4]. The AI-algorithm was fine-tuned on small collections of RXR activating natural products. It generated synthetically accessible NCEs populating an unexplored chemical space at the interface between drug-like molecules (used for training) and natural products[4] (used for fine-tuning). Again, top-ranked computational samples were synthesized and two out of four were active in vitro with similar potencies (EC50 16 - 27 µM) as the fine-tuning set (EC50 2.1 - 43 µM) confirming that the designs inherited the bioactivity profile of the natural product templates.

Our results highlight generative AI as valuable data-driven tool for medicinal chemistry to obtain synthetically accessible and innovative NCEs[5] that inherit properties and bioactivity of a template collection without the need of explicitly including molecule design rules.

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2.3 Laboratory Automation

Chair: H. Wätzig

SL.08

Important features of lab automation: an overview

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In the course of lab 4.0 automation and digitalization in pharmaceutical laboratories increases productivity in the labs. An explorative field study at the Analytica in Munich 2018 and the Achema in Frankfurt 2018 shows different options to automate processes. Based on an interview guideline for vendors 50 companies were interviewed. For example, Andrew Alliance, Axel Semrau, Brand, Zinsser, Tecan, Eppendorf and Beckman Coulter produce very different types of liquid handling systems. It depends on the need and wishes of the customers. Some companies provide robots for solid handling and tablet dissolution tests. The software is developed in two different ways: open/flexible for programing vs. user-friendly/ intuitive /drag and drop. Usability studies are highly recommended to get a good overview about the wishes and requirements of the customers.

The most common automation technique is the liquid handling. It is applied by High Throughtput Screening (HTS), Next Generation Sequenzing (NGS) and for proteomics and genomics in general.

In this project, the manual and automatic sample preparation of complex samples with the liquid handler of Zinsser and Brand were compared.

The most important advantages of automation are the higher reproducibility and reliability in sample preparation and data evaluation, because every lab worker prepared the samples on his own way despite Standard Operation Procedures (SOPs). Furthermore, the sample tracking is mostly included. That is important for the supervisory authorities like FDA and EMA controlling your process.

For establishing automation in a pharmaceutical company, the previous manual process and the new automatic one should be compared. They have to deliver the same results. The guidelines for the process validation include the Good Automated Manufacturing Practice (GAMP), ICH Q2 and FDA CFR11.

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SL.09

Robotic lab automation; state of the art and prospects

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XYZ robotic systems offer a high degree of automation and are nowadays used in nearly every lab.

Starting around 20 years ago with autosamplers for liquid injection only, today also more or less complex sample preparation steps like standard addition, derivatization or protein digestion are possible on modern chromatography workstations. Online coupling to high end detectors and active communication between all used analytical components became standard to reach short turnaround times. Coupling to other analytical instruments like balances, photometers, shakes, ultrasonic baths…., is possible.

For comfortable use of these workstations also an intelligent software solution is needed that provides ideally only one sample list, no matter how many instruments are combined. Intuitive control, possibility of overlapped sample preparation or combination of different methods within one run, security and documentation and compatibility to all established chromatography systems is also a must to reach the highest comfort and acceptance.

Main advantages of these workstations are prevention of error sources due to manual sample preparation, high level of automation without manual intervention, high sample throughput, flexibility, no risk of contamination and an excellent and calculatable reproducibility.

Still, the working area is spatially and technically limited making many working steps not automatable. So multidimensional robots, already know from industrial use, are the next step and the optimal addition to XYZ robotic systems to take over further tasks. Six independently movable joints make them more flexible and suitable for various workflows. The fields of application are manifold and are defined by user requirements.

SL.10

Robotics as a tool for the 21st century biology

P. Zucchelli

The discovery of DNA created the conditions for conscious editing of the basic code of living beings - and we are just at the beginning. What will happen in the coming century? and which tools will allow it to come to practice and impact everybody? We present the idea that robotics will be the enabling tool for the collection of genetic information, but also the solution for its democratic exploitation in the pharmaceutical and medical industry.

2.4 cGMP-signalling

Chairs: J. Schloßmann, P. Ruth

SL.11

Cardioprotection by cGMP and its signal transduction to mitochondria

R. Lukowski

Abstract not available.

SL.12

Physiological effects of cGMP in heart muscle cells – a matter of compartmentation

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3',5'-cyclic guanosine monophosphate (cGMP) is an ubiquitous second messenger that regulates cardiac function by acting in a compartmentalized manner in distinct subcellular microdomains. Functionally important microdomains are formed around calcium handling proteins such as membrane calcium channels, calcium reuptake channels at the sarcoplasmic reticulum, and in microdomains formed around the sarcomeres. Local microdomain-specific cGMP dynamics which is regulated by anchored pools of kinases and cyclic nucleotide degrading enzymes phosphodiestarases (PDEs) has not been extensively studied before. Using cytosolic and targeted fluorescent biosensors expressed in transgenic mice to monitor cGMP in such microdomains, we recently determined exact membrane localization of cGMP producing natriuretic peptide receptors guanylyl cyclases A and B (GC-A and GC-B) and analyzed compartmentation of their intracellular signals. We used scanning ion conductance microscopy (SICM) combined with Förster resonance energy transfer (FRET) based cGMP biosensors to show that while GC-B (receptor for C-type natriuretic peptide, CNP) is uniformly localized on the cardiomyocyte membrane, functional GC-A receptors (for atrial natriuretic peptide, ANP) are found exclusively in membrane invaginations called transverse (T)-tubules. Although both ANP and CNP protect from cardiac hypertrophy, their effects on contractility are markedly different, from almost no effect (ANP) to strong negative inotropic and positive lusitropic responses (CNP) with unclear underlying mechanisms. Using out real time imaging techniques, we could show that uniform CNP membrane localization leads to CNP/GC-B/cGMP signals which diffuse over long distances inside the cell, whereas ANP/GC-A/cGMP signals are highly confined to T-tubular microdomains by local pools of PDE2 activity. This provides a clear molecular basis and explains distinct functional effects engaged by the two natriuretic receptors and their respective pools of cGMP in the heart.

SL.13

cGMP-dependent signaling pathways in chronic pain processing

Jonas Petersen [1], Evanthia Mergia [2], Lea Kennel [1], Oliver Drees [3], Rebecca Steubing [3], Catherine Isabell Real [3], Wiebke Kallenborn-Gerhardt [1], Ruirui Lu [1], Andreas Friebe [4], Doris Koesling [2], Achim Schmidtko [1]

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A large body of evidence indicates that nitric oxide (NO)/cGMP signaling essentially contributes to the processing of chronic pain. In general, NO-induced cGMP formation is catalyzed by two isoforms of guanylyl cyclase, NO-sensitive guanylyl cyclase 1 (NO-GC1) and 2 (NO-GC2). However, the specific functions of the two isoforms in pain processing remain elusive. Here we investigated the distribution of NO-GC1 and NO-GC2 in pain-relevant tissues, and we characterized the behavior of mice lacking either isoform in animal models of pain. Using immunohistochemistry and *in situ* hybridization we demonstrate that both isoforms are distinctly localized to interneurons in the spinal cord and to non-neuronal cells in dorsal root ganglia. Mice lacking NO-GC1 or NO-GC2 demonstrated specific phenotypes in models of neuropathic and inflammatory pain, pointing to pronociceptive and antinociceptive functions of cGMP. Cre-mediated deletion of NO-GC1 or NO-GC2 in spinal cord neurons recapitulated the behavioral phenotypes observed in the global knockout. Together, these results indicate that cGMP produced by NO-GC1 or NO-GC2 in the spinal cord exerts distinct, and partly opposing, functions in chronic pain processing.

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2.5 New Research, New Researchers

Chair: S. Laufer

SL.14

Confocal Raman microspectroscopy as an alternative method to characterize stratum corneum lipid content and conformation

Ziwei Zhang, Dominique Lunter

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Emulsifiers are frequently used in topical formulations like emulsions and creams. Their main task is to stabilize the dispersed phase in the coherent phase against coalescence during storage. Upon application to the skin, emulsifiers may interact with it. Especially the outermost layers of the skin, the stratum corneum (SC) may be affected. The SC consists of keratinocytes surrounded by layers of highly ordered lipids which are responsible for maintaining the excellent barrier function of the skin. Interaction of emulsifiers with the SC lipids bears the risk of impairing this barrier function. Especially when formulations are applied to already impaired skin e. g. due to a skin disease, such impairment of the barrier properties should be avoided. It is thus of pivotal importance to characterize emulsifiers with regard to their effect on SC lipids. This is usually done with methods such as high-performance thin-layer chromatography (HPTLC), differential scanning calorimetry (DSC) or histological staining. These are time consuming and often rely on extensive pre-treatment of the samples or are destructive. Confocal Raman microspectrosopy (CRM) offers a non-destructive alternative that needs no or only little sample pre-treatment. The purpose of this study was thus to investigate the impact of emulsifiers on intercellular lipids of porcine SC and to evaluate CRM as an alternative method in this research context. To this end, emulsifiers that are typically used in semisolid preparations were tested in aqueous solution/dispersion in the respective concentrations as present in typical formulations. CRM was used to determine lipid content and conformation in the emulsifiers treated SC. Results were correlated to results from the conventional methods: HPTLC (lipids content), DSC (lipids conformation) and histological staining (SC thickness). Various emulsifiers and emulsifier mixtures showed different impact on SC lipid content, conformation and SC thickness. Emulsifiers and their mixtures that reduced the lipids content also reduced SC thickness and shifted lipids conformation to less ordered state. This indicates that lipid extraction and disorder are the reason for SC thinning. Results from CRM and conventional methods showed a strong correlation for both lipid content, conformation and SC thickness measurements [1, 2]. With easy sample preparation and fast analytical readout, CRM has the potential to be a standardized analytical method for skin lipids investigation.

PD Dr. Martin Schenk is acknowledged for the donation of pig ears. This project was supported by the European Social Fund and by the Ministry of Science, Research and the Arts Baden-Wuerttemberg and the China Scholarship Council.

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SL.15

Nanomechanics of fibroblast growth factor 2 – heparin at physiological and pathophysiological conditions: Implications for drug delivery

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Fibroblast growth factor 2 (FGF2) – an important potent mitogenic and paracrine growth factor – binds electrostatically with low micro-molar affinity to heparan sulfates present on extracellular matrix proteins [1]. FGF-2 is spatially and temporarily tightly regulated during embryonal development and in tissue repair, showing proliferative effects on a large number of cell types derived from mesodermal and ectodermal origin. This study aimed at investigating the force landscape of heparin – a heparan sulfate surrogate – and FGF2 at the molecular level to elucidate binding characteristics at physiological and pathophysiological states, in which a stressed cellular microenvironment was mimicked by lowering the extracellular pH-value as result of hypoxia (low pO_2 levels) as found in wound sites, inflammation, or cancer.

Biomolecular pulling as well as force clamp atomic force microscope (AFM) experiments using the method of microparticle-based magnetic actuators between low molecular weight heparin and FGF2 interaction at the molecular level were performed [2, 3]. Unbinding forces and bond lifetimes between both interaction partners in a physiological state (pH 7.4) and at pathophysiological conditions (pH 5.5) were recorded and analyzed in line with isothermal titration calorimetric experiments.

Unbinding events between FGF2–heparin complexes were specific and short-lived (~ 0.41 sec). Binding between FGF2 and heparin had strong slip bond characteristics as demonstrated by a decrease of lifetime to ~ 0.19 sec with tensile force on the complex. An acidic pH environment (5.5) modulated FGF2 – heparin binding as demonstrated by enhanced rupture forces needed to release FGF2 from heparin-FGF2 complex and as compared to physiological conditions.

Our data provide first nanomechanical insights into the FGF2 at the molecular level with a modular AFM system designed for biomolecular experiments. Solution pH modulated FGF2 – heparin binding and release as demonstrated by enhanced rupture forces at acidic conditions compared to physiological pH. These molecular insights form an intriguing starting point for hypothesis building on disease-guided FGF-2 release and as a blueprint for the biomimetic design of novel drug delivery systems for heparan sulfate interacting growth factors in the future.

Acknowledgments: Funding from the FET Open FP7 European project MANAQA (Magnetic Nano Actuators for Quantitative Analysis, 296679) is gratefully acknowledged.

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SL.16

The rapid onset of plasma concentrations after oral administration of a novel Aspirin® formulation in the fed state can be explained by the presence of the Magenstraße

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Acetylsalicylic acid (Aspirin®) is available in various oral dosage forms that possess different pharmacokinetic (PK) properties [1]. Recently, a novel Aspirin® formulation (Aspirin® 500 mg Überzogene Tabletten, Bayer AG, Germany) was introduced that shall provide fast drug absorption in order to treat various forms of pain quickly and efficiently. This novel formulation is based on micronization of the drug and addition of sodium carbonate as an effective disintegrant. In a recent PK study, the pharmacokinetic properties of the novel formulation were compared with two other immediate release formulations of Aspirin® (i.e. dry granules and conventional tablet) in the fasted state. This study nicely showed that the maximum concentrations of acetylsalicylic acid as well as salicyl acid were higher for the novel formulation and that t_{max} was also significantly shorter (17.5 min vs. 45 min for the tablet) [2]. This beneficial pharmacokinetic behaviour results in faster analgesic efficacy [3] and is probably caused by two effects: the rapid dissolution of the drug as a result of the micronization of the drug and the addition of carbonate as an effective disintegrant as well as the acceleration of gastric emptying by the generation of carbon dioxide.

Recently, the novel Aspirin® formulation was also characterized in a fed state PK study in comparison to the old formulation. Surprisingly, t_{max} was not affected by the intake of food. This effect was assumed to be caused by the presence of the Magenstrasse (stomach road), a physiological mechanism allowing the rapid emptying of water from the fed stomach [4].

It was the aim of this study to investigate drug release from the novel Aspirin® formulation under fed conditions with the aid of a novel biorelevant in vitro dissolution test device – the GastroDuo. This device allows the simulation of dynamic changes of gastric pH and temperature, realistic gastric emptying kinetics as well as of gastric peristalsis. The pH profiles and gastric emptying rates simulated in this study were based on recent in vivo data on the FDA standard breakfast that were obtained by using the telemetric SmartPill® system and magnetic resonance imaging [4,5].

The in vitro experiments clearly showed that disintegration and dissolution of the novel Aspirin formulation was faster compared to the conventional tablet formulation under physiologically relevant conditions. The experiments with the GastroDuo further revealed that this accelerated drug dissolution allowed the rapid transfer of dissolved drug to the small intestine. This observation confirmed the hypothesis that the rapid onset of plasma concentrations was caused by the presence of the Magenstrasse in the fed stomach.

The data presented in this study demonstrate that the Magenstrasse represents an interesting physiological phenomenon that can be used to enable rapid drug absorption of drugs after administration together with food. This concept can be valuable for oral pharmacotherapies, in which a fast onset of plasma concentrations is needed.

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SL.17

A multimaterial system for on-demand protein release

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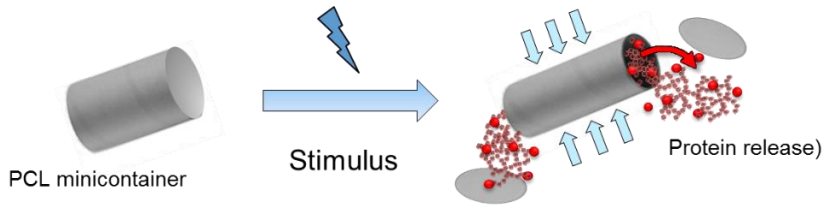
In order to control tissue regeneration processes, a local delivery of therapeutics such as protein drugs can be advantageous, particularly when the time point of release and release profile can be controlled. For precision medicine, it may further be necessary to choose defined protein drugs depending on the clinical picture during a surgical or minimally invasive intervention. Those compounds may be immediately placed during the intervention in a release system that allows a later on-set of release depending on the healing progress. This calls for a degradable device that is flexible in terms of type of compound to be loaded, allows an on-demand control of the release, and potentially is even compatible with an intraoperative drug incorporation procedure.

Here, a multifunctional multimaterial system for on-demand protein release is presented, which was demonstrated to be applicable for various types of proteins in vitro [1]. This system composed of mini-tube devices from poly(ε-caprolactone) networks, which were programmed to show a shape-memory effect, which can be an on-demand shrinkage resulting in reduced diameters. This shape-memory effect could be triggered either by elevated temperature or tissue-permeable NIR-light. By incorporating the desired proteins in the internal lumen of the tubular devices, either as pure solution or a PLGA-PEG-PLGA gel formulation, the decrease of diameter of these heat-shrinkable tubes allowed sequentially coupling diameter reduction and expelling of payload from the lumen.



With this system, different release options were enabled: Using open tubes containing proteins in a gel formulation, a sustained linear initial release with a triggered add-on dosing was demonstrated. When the orifices of the tubes were sealed (see Figure), a negligible initial release and an on-demand onset of bolus (protein solution) or sustained release (hydrogel formulation) were shown. As exemplified for model proteins of different molecular weights, this system is applicable to various proteins showing a very comparable release profile independent from their molecular weight. Furthermore, as analyzed for SDF-1α, a therapeutically relevant chemotactic protein, the release could be triggered at different time points as desired.

Acknowledgments: Technical support by Mrs. Pfeiffer, Mrs. Schwanz, Dr. Ziegler, Mrs. Pieper, and Mr. Stoermann is acknowledged. The NIR light irradiation set-up was acquired with support of the German Research Foundation (grant # WI 3637/1-1). Funding for this study was provided by the Federal Ministry of Education and Research, Germany, in the Program Health Research (grant No. 1315848B and 13GW0098) and by the Helmholtz Association through programme-oriented funding.

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SL.18

Polysorbate degradation in biotherapeutic formulations: Identification and discussion of current root causes.

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Biotherapeutic protein formulations are often high concentration liquid protein solutions, which are required to be stable under pharmaceutically relevant storage conditions and presence of external stress. Non-ionic detergents like Polysorbate have been the most commonly used excipient to maintain formulation stability[1]. Recently, particle formation in Polysorbate containing biotherapeutic formulations has arisen as a major quality concern and patient risk factor[2]. During this study we explore different mechanisms and pathways leading to Polysorbate degradation. Specifically, oxidative degradation and hydrolytic (chemical and enzymatic) degradation has been investigated. For this purpose, an RP-HPLC based Polysorbate analytical method was employed along with fluorescence micelle assay. Additionally, free fatty acid contents were monitored during Polysorbate degradation. We could show that different degradation pathways have unique degradation 'fingerprint' which can be used as a tool to assess the degradation in biopharmaceutical formulations.

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2.6 3D(ifferent) Views of Antibiotic Resistance

Chairs: P. Heisig, A. Heisig

SL.19

David versus Goliath: Ribosome-target antibiotics and bacterial resistance mechanisms.

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The ribosome is the protein synthesizing machine of the cell and one of the main antibiotic targets in the bacterial cell [1-3]. Structures of naturally produced antibiotics and their semi-synthetic derivatives bound to ribosomal particles have provided unparalleled insight into their mechanisms of action [1-3]. In this presentation, I will discuss the recent structural insights into the mechanism of action of ribosome-targeting antibiotics as well as the mechanisms of bacterial resistance.

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SL.20

3D(ifferent) Views of Antibiotic Resistance – the Cellular View: Bacterial Strategies to Develop High-Level Fluoroquinolone Resistance

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According to WHO antibiotic-resistant bacteria are one of the most urgent health problems worldwide due to both, an increasing prevalence of mutiple drug resistant (mdr) isolates of relevant pathogenic species and to the drying pipeline of novel antibiotic compounds with enhanced antibacterial activity.

Clinically relevant resistance to antibiotics of natural origin, e.g. -lactams, often is based upon the acquisition of one or more genes encoding drug-inactivating enzymes, such as -lactamases, located on transferable plasmids, the development of resistance to non-naturally occurring antibiotics such as the fluoroquinolones predominantly affects chromocomally encoded genes. Using Escherichia coli (E.coli) as a model microorganism of high clinical relevance and fluoroquinolones as the most relevant antibiotic class of non-natural origin the bacterial strategies to resist the bactericidal acivity of these drugs were studied on the molecular genetic level.

The data presented will provide a more detailed view of the development of highly-resistant pathogens as a complex multiple-step process which is not simply a combination of mutations directly associated with fluoroquinolone resistance, but in addition involves mutations in genes encoding non-resistance-associated functions which are involved in different metabolic processes.

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SL.21

3D(ifferent): Views of Antibiotic Resistance: The environmental view

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Multi-resistant bacterial pathogens have become a worldwide threat to public health [1, 2]. Their success has been ongoing for two decades and provoked the appearance of multidrug resistant (MDR)-bacteria and antimicrobial resistance (AMR) in the WHO Top Ten list of major future health complications [3]. An ubiquitous occurrence of pan-resistant bugs and the lack of efficient antimicrobial substances to fight them will not only have severe effects on infection medicine, as modern healthcare relies on antimicrobials that will become ineffective. This is especially true for Gram-negatives which have been constantly increasing within the last decades in Europe and all over the world[4]. Besides their emergence in human medicine, the same MDRs have also become abundant in the environment all over the world.

Prime examples for this development are Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumonia,* so-called "high risk clones", which present highly clonally related bacteria[2] of one species. They share the same phylogenetic background and sometimes present a fixed combination with a large MDR-plasmid (O25:H4-ST131-CTX-M15 or O1:H6-ST648-CTX- among others such as ST617, ST167, ST410, ST224 and ST117)[5, 6]. These strains are not only extremely successful in clinical contexts but also in extra-clinical settings such as the community, wildlife and remote environments, even in the absence of a constant antimicrobial selection pressure[7-9].

So what is the reason for the occurrence of successful MDR-lineages in different environments although these habitats lack the dogmatic prerequisite of "antimicrobial selection pressure"? The old dogma that harboring large ESBL-plasmids would mean fitness costs to the host bacterium and the lack of a selective pressure would lead to a loss of the plasmid and/or out-competition of the resistant population by a sensitive one is contradictory to the recent spread of "high risk clones" in the environment. Current data on high risk clones suggests that

i) there is no fitness loss due to the carriage of ESBL-plasmids in successful clonal lineages; and

ii) factors other than resistance such virulence contribute substantially to the spread of ESBL-*E. coli.*

Both hypothesis presented a paradigm shift in the field of antimicrobial resistance research, which has concentrated on resistance genes itself for decades. The dogma of resistance costs for the bacterial host is not valid for ESBL-producing high risk clones of *E. coli*, and plasmid carriage can even provide selective advantages beyond resistances such as biofilm formation, important for virulence.

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2.7 New Research, New Researchers II

Chair: A. Link

SL.22

Connecting lysosomes and mitochondria in cancer - regulation of lipid metabolism and mitochondrial function by the V-ATPase

Bartel K.[1], Schmitt S.[2], Koeberle A.[3], Menche D.[4], Werz O.[3], Müller R.[5], Zischka H.[2], Vollmar A.M.[1], Schulze A.[3], von Schwarzenberg K.[1]

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Cellular homeostasis is important for maintaining proper cell function to enable cell growth and proliferation. In recent years the lysosomes, which have long been simply regarded as the cells degradation compartment, have been discovered as essential regulators of cellular homeostasis. They are the central recycling stations of the cell, thereby regulating processes like cell death induction, autophagy and nutrient sensing, processes that are frequently deregulated in cancer. Hence, lysosomes have gained great interest as target in novel anti-cancer treatment strategies.

In previous work, we demonstrated a link between lysosomal regulation of cholesterol homeostasis and cancer cell proliferation [1]. Using Archazolid, a highly selective and potent inhibitor of the lysosomal vacuolar-H+ATPase (V-ATPase), we further investigated the role of lysosomes in cellular lipid regulation. We found evidence, that disrupting lysosomal function leads to alterations in cancer cell lipid regulation. Upon treatment, cancer cells increase CD36 dependent lipid uptake and upregulate de novo lipid synthesis via the PGC1α-PPARα-axis. Furthermore, intracellular triacylglycerol distribution and composition is altered upon V-ATPase inhibition. As revealed by lipidomics data, TAG contain a higher proportion of saturated fatty acids. Lipid droplets, the lipid storage organelles, are reduced in number and size, while the amount of free fatty acids in the cytoplasm increases. Besides serving as structural molecules for the building of membranes, an essential function of the cellular lipid pool is maintenance of mitochondrial energy generation and regulation of reactive oxygen species (ROS) generation. We found that the elevated amount of free fatty acids is accompanied by reduced NADPH levels and leads to an impairment of mitochondrial function. V-ATPase inhibition leads to morphological and structural changes in mitochondria, displaying a fission phenotype. Analysis of mitochondrial oxygen consumption by the seahorse technology shows a reduction of mitochondrial respiration and a shift in mitochondrial fuel dependency. These changes lead to a loss of mitochondrial membrane potential and an increase in cellular ROS levels, which is linked to cell death.

Our study, provides first insight into a complex inter-organelle cross-talk between lysosomes and mitochondria in cancer cells, that is influenced by changes in cellular lipid regulation. Our findings will help to develop new anti-cancer treatment strategies in the future.

This work was supported by the DFG research funds 1406 SCHW 1781/1-1 and AV 376/18-1

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SL.23

Phyllobilins – An Underexplored Family of Natural Products that have an Effect on Cancer Cells

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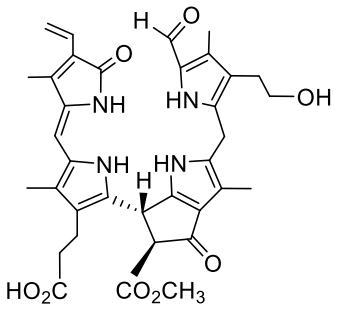
Department of Pharmacy, Pharmaceutical Biology, Ludwig-Maximilians-University of Munich



Phylloxanthobilin

The focus of our studies is on a family of plant derived natural products, the phyllobilins. These tetrapyrrolic compounds arise from the degradation of chlorophyll and are therefore ubiquitous in nature. Phyllobilins have been discovered roughly 25 years ago and have since then been detected in leaves, the peels of ripening fruit, and vegetables.[1,2] Despite their abundance and the fact that they are part of human nutrition, phyllobilins are surprisingly unexplored in regard to their physiological properties.[3] The main phyllobilins are colorless and can be oxidized to yellow chlorophyll catabolites (phylloxanthobilins) that were also shown to occur naturally.[4] In our studies, we found that a phylloxanthobilin had significant effects on cancer cells; the compound inhibited proliferation in the micromolar range and was able to induce apoptosis in a dose dependent manner. We further aim at understanding the mechanism by which the phyllobilins kill cancer cells and plan to employ chemical synthesis for derivatization of the natural products to further improve their activities.

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SL.24

Avoiding depurination in the synthesis of DNA-tagged tetrahydroquinolines by micellar Brønsted acid catalysis

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Methods for reacting DNA-tagged small organic molecules to target compounds are sought not the least since genetically encoded compound collections called DNA-encoded libraries (DELs) have found broad use as screening technology [1]. Encoded compound synthesis must take into account the chemical lability of DNA, for instance ready cleavage of purines from the oligomer under even mild acidic conditions. Thus, only a very restricted set of synthesis methods can be employed in DEL synthesis [2]. We have developed a DNA-tagging strategy that employs a chemically very stable hexathymidine sequence in the first step of encoded library synthesis [3,4]. It allows for initiation of DEL synthesis with a broad spectrum of synthesis methods.

Compartmentation of reaction media holds in principle much promise for encoded library synthesis. Compartmentation is usually effected by amphiphilic molecules. These can be designed to form oil-in-water micelles in water spontaneously, and at low concentration. Sulfonic acid-conjugated amphiphile polymers confined the acid moiety to the DNA-inaccessible oil phase (figure 1). DNA strands can be incubated for prolonged time with high concentrations of these acid nano reactors with impunity. DNA-tagged aldehydes are converted by micelle-catalyzed Povarov reaction readily and with no detectable depurination to diverse substituted tetrahydroquinolines, a frequently occurring scaffold in bioactive compounds [5].[5] We found the polymer catalyst design to be crucial for successful heterocycle formation and protection of DNA.

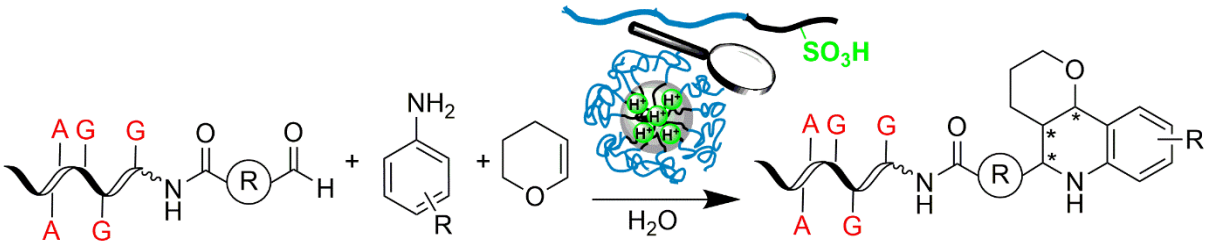

Figure 1. Amphiphile polymers with sulfonic acid moieties immobilized in the lipophilic part, enable the Brønsted acid-mediated synthesis of DNA-tagged tetrahydroquinolines from aldehydes, anilines, and olefins.

We are grateful for financial support by the German Federal Ministry of Education and Research (BMBF) Grant 1316053, and the Mercator Research Center Ruhr Grant Pr-2016-0010.

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SL.25

The thermodynamics of elastin-like protein (ELP) assembly: a circular dichroism-based approach

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Chiroptical spectroscopy techniques such as circular dichroism (CD), vibrational circular dichroism (VCD), and Raman optical activity (ROA) are highly sensitive toward changes in secondary or higher-order structure. In addition to providing qualitative information about conformational states (for example the presence of α-helix or β-sheet structure), a chiroptical spectrum can also be evaluated quantitatively through the application of global fitting techniques. The particular advantage of this approach is that it is non-invasive and does not pose a manipulation on a thermodynamic equilibrium.

Previously, we have analyzed the quantitative response on pH,[1] chemical modification,[2] and a time-dependent process[3] by fitting physical models to chiroptical spectra. Modelling temperature-dependent processes is generally more challenging, as temperature both affects the thermodynamic equilibrium and the kinetics of a structural transition. For the conformational changes of an elastin-like protein (ELP), however, we found a good correlation with a pure thermodynamic model being in agreement with an entropy-driven folding process (Figure 1). The understanding of these processes is highly relevant, as temperature-controlled assembly might have a role in the generation of motion in elastin containing tissues.[4] Furthermore, the assembly of ELPs can be employed to obtain organelle-like lipid-free nano-compartments.[5]

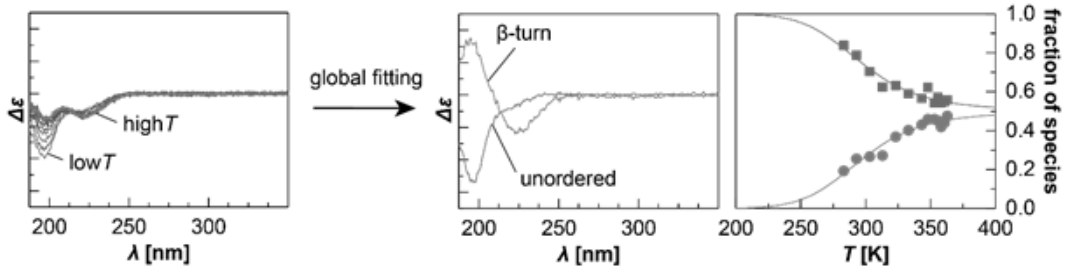

Figure 1. Temperature-dependent changes in the CD spectrum of an elastin-like protein reflect the entropy-driven transition from unordered to β-turn structure.

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2.8 Pharmacometrics for in vitro models, mice, and men

Chairs: C. Kloft, S. Wicha

SL.26

Human PK prediction – a generic approach combining both in vitro and in vivo preclinical data

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Introduction – The human dose prediction, which is typically first performed already during the research and drug discovery phase, is very important (nevertheless still challenging). The result is fundamental to evaluate important drug and/or target characteristics early on, such as the evaluation of the therapeutic window, potential dosing regimens and to adequately plan the clinical development program for a drug candidate. The human dose prediction can be split into two parts – defining efficacious target exposure as well as predicting the human pharmacokinetics (PK). The latter includes predicting the volume of distribution at steady state (VSS), the clearance (CL), but also additional PK parameters relevant to adequately anticipate the shape of the human plasma concentration-time profile (e.g., number of PK compartments, central versus peripheral volume of distribution). Various methods have been published to estimate these parameters based on in vitro and/or in vivo data. These methods range from purely empirical methods to mechanistic physiologically-based PK (PBPK) models [1]. For example, methods to predict the clearance range from single species scaling (assuming the same clearance per kilogram body weight), or allometric approaches to in vitro in vivo correlation (IVIVC)-based prediction methods [2]. However, no single method can adequately predict the relevant PK parameters for all drug candidates. Furthermore, most of the methods only include a small part of the preclinically available in vitro and in vivo data. The objective of this presentation is therefore to describe a medium to high throughput workflow to make optimal use of the available preclinical in vitro and in vivo data.

Discussion & Example – One of the most important aspects and the aim of a human PK prediction should be to gain a sound understanding of the human PK processes. For example, the CL as one of the most important PK parameters represents the systemic drug elimination. It typically consists of multiple elimination processes of varying importance. Some drugs are mainly renally eliminated, whereas other drugs are predominantly eliminated by hepatic metabolism or excretion into the bile. For drugs, which are mainly eliminated via hepatic metabolism, an IVIVC between the in vivo clearance estimate and the in vitro hepatocyte clearance for multiple preclinical species should be investigated as a first step. A poor quality of this correlation can be a hint for relevant renal elimination or excretion into bile, which would not be captured by the clearance estimate based on hepatocyte clearance. Therefore as a second step, these processes might be well-captured by an allometric scaling approach. If a relevant fraction of the drug is renally eliminated, the hepatic clearance and the renal clearance should be extrapolated separately from the preclinical species (including at least one non-rodent species) to man. The renal elimination is the sum of the glomerular filtration rate (GFR) and active secretion into urine, but reduced by reabsorption from the urine to plasma. Dependent on the relevance of active secretion into urine, it might be more adequate to predict the human renal clearance by incorporating either the between species differences in the GFR or the kidney blood flow. When the GFR is the main renal elimination mechanism, it is essential to not only scale for differences in the perfusion, but also to incorporate species differences in plasma protein binding. The presentation will include an example, which illustrates this workflow not only for the clearance parameter but also for all other relevant parameters described in the introduction. However, it has to be emphasized that while this work flow generally provides a reasonable way to predict the human PK, drug-specific and species-dependent aspects should always be additionally investigated and if necessary, the workflow should be adapted accordingly.

Conclusion – To adequately predict the human PK, as a fundamental part of the human dose prediction, it is mandatory to include both in vitro and in vivo data. While a single method to predict e.g. the human clearance might be adequate for drugs that are either cleared by metabolism or renal elimination, a workflow including different prediction methodologies is required to perform good human PK predictions for the majority of drug candidates.

Acknowledgments: The presentation includes parts of the aligned human dose prediction strategy applied at Boehringer Ingelheim. All who contributed developing this strategy are gratefully acknowledged.

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SL.27

Translational PK/PD modelling of ISTH0036, a potent and selective antisense oligonucleotide targeting transforming growth factor beta 2 (TGF-β2) for the treatment of ophthalmic diseases

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In ophthalmology, several diseases have been linked to the modulation of transforming growth factor beta (TGF-β) expression. Specifically for TGF-β2, a critical role in the pathophysiology of glaucoma and retinal disease has been demonstrated, making this isoform a relevant therapeutic target for a disease which is the leading cause for irreversible blindness in the world. ISTH0036 is an antisense oligonucleotides (ASO) which is currently in clinical development. This presentation will summarize the translation preclinical pharmacokinetic/pharmacodynamics (PK/PD) modelling approach to support clinical drug development.

ISTH0036 was administered in vivo via intravitreal (IVT) injection to the eyes of New Zealand (albino) rabbits as single and multiple dose administration. Ocular tissues were analyzed for tissue drug concentrations and target mRNA downregulation. First, a PK model of the ISTH0036 concentration time profiles in vitreous humor, aqueous humor, cornea, lens, sclera, optic nerve, choroid/retina and ciliary body/Iris was established. Second, tissue concentrations were linked to the respective mRNA expression levels (PD). Third, the final PK/PD model was used to predict various administration protocols to guide clinical drug development. PK/PD modelling was performed using NONMEM 7.3. Statistics and graphs are generated using SAS 9.4.

ISTH0036 eye plasma concentration-time profiles in the eye were well described by a 14-compartment model, where all tissues, except cornea and aqueous humor required a peripheral compartment for adequate description of the PK. Lens, sclera, optic nerve, choroid/retina, ciliary body/Iris and aqueous humor were connected to the vitreous humor compartment; cornea was connected to the aqueous humor compartment. ISTH0036 was eliminated via Michaelis-Menten kinetics from the vitreous and aqueous humor compartment. TGF-β2 mRNA expression was best described by a turn-over model with zero-order synthesis and first-order elimination processes in tissues. The synthesis rate was reduced by the ISTH0036 concentrations in the peripheral compartment using an Emax model, where EC50 and Emax values varied depending on the tissue investigated. For the prediction of human PK, various dosing regimens were tested (every 4, 8, or 12 weeks) and the mRNA expression in the respective tissues was simulated. A dosing regimen every 4 weeks showed a faster steady-state and a more pronounced reduction in TGF-β2 mRNA expression across all tissues investigated.

A comprehensive PK model was established to describe the complex tissue distribution of ISTH0036 into the the eye after single and multiple dose administrations. The PK model was successfully extended by the description of the potent target TGF-β2 mRNA downregulation in relevant tissues of the rabbit eye. The PK/PD model developed in rabbits was successfully applied to guide clinical development of ISTH0036.

SL.28

Pharmacokinetic and pharmacodynamic studies aiming for rational drug dosing in preterm neonates: the DINO study

S. Völler[1,2], R.B. Flint[2,3,4], R. de Groot[5], K.D. Liem[6], P. Andriessen[7], P.L.J. Degraeuwe[8], I. Reiss[2], D. Burger[3], D. Tibboel[9], C.A.J. Knibbe[1,10], S.H.P. Simons[2] and the DINO Research group

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Background and Aim

The DINO-study (Drug dosage Improvement in preterm NeOnates) simultaneously collected pharmacokinetic and pharmacodynamic data of nine frequently used drugs, namely Doxapram, Fentanyl, Levetiracetam, Midazolam, Paracetamol, Phenobarbital, Sildenafil, Fluconazole and Ibuprofen in preterm neonates. Seven of these drugs are still used off-label. It aims to increase safety and efficacy by improving dosing recommendations in this vulnerable population.

Methods

By using sparse opportunistic blood sampling using limited sample volumes of 200 µL, the burden to the individual child is minimal. The study was performed in 4 centres in the Netherlands, enabling the DINO team to collect more than 1500 samples. In about one third of these samples more than one drug can be quantified as children received multiple study drugs simultaneously. The development of assays with a sample volume of 10-50 µL allows for the use of all these data.

Results

Population pharmacokinetic models are being developed using non-linear mixed effects modelling. Pharmacodynamics are investigated through effect measurements such as pain scores or second-to-second monitor read-outs of oxygen saturation to relate the concentration to a certain effect. This model-based approach enables us to evaluate current dosing advises and to propose new dosing regimen where necessary. Thereby, it aims at substantially increasing the proportion of new-borns in the therapeutic window, potentially resulting in higher success rates of treatment and less side-effects.

Conclusion

The design of the DINO-study serves as a suitable example on how to perform pharmacokinetic-pharmacodynamic research in preterm infants and can be extended to the whole pediatric research field. Current collaborations will enable us to use the network for future research, such as the prospective evaluation of the developed dosing recommendations or for new drugs.

2.9 Gene Therapy

Chairs: E. Oetjen, L. Carrier

SL.29

Gene supplementation therapy for CNGA3-linked achromatopsia

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Achromatopsia is a clinically well-defined inherited retinal disorder characterized by day blindness, poor visual acuity, photophobia, nystagmus, and lack the ability to discriminate colours. About 95 % of the patients carry loss-of-function mutations in either the CNGA3 or the CNGB3 subunit of the cone photoreceptor cyclic nucleotide-gated cation (CNG) channel. Previously, we have established a murine model for CNGA3-linked achromatopsia. Importantly, in this preclinical model we were able to restore impaired cone-mediated vision using AAV (adeno-associated virus) -mediated gene supplementation. Based on this work we have designed AAV8.CNGA3, a recombinant AAV vector for gene supplementation therapy of human CNGA3-linked achromatopsia (ACHM2). The vector expresses human CNGA3 under control of a short human arrestin 3 promoter and was packaged with AAV8 capsid. A first-in-man dose escalation clinical trial with nine ACHM2 patients (NCT02610582) was conducted focusing on safety and efficacy of a single subretinal injection of AAV8.CNGA3. Three patients were treated per dose group (0.1-1.0 x 10e11 total vector genomes). Safety as primary endpoint was assessed by clinical examination of ocular inflammation. The primary endpoint was met with an excellent safety profile and no serious adverse event. Secondary outcomes were change in visual function from baseline in terms of spatial and temporal resolution, chromatic-, luminance-, and contrast sensitivity over a period of 12 months post treatment. Analysis of secondary endpoints supports the notion that the treatment improved clinical features in nine out of nine patients. In conclusion, the first clinical gene therapy trial for achromatopsia in man was well tolerated, safe and provides evidence that cone photoreceptors in ACHM2 can be reactivated by gene supplementation.

SL.30

Gene Therapy - limitations of viral gene transfer and possible solutions

M. Trepel

Abstract not available.

SL.31

Endothelium as a target for the gene therapy of brain diseases

Markus Schwaninger

Until today, no efficient treatment is available for numerous genetic and multifactorial disorders of the CNS. In this situation, gene therapy may open new therapeutic avenues. However, the need to navigate gene vectors through the blood-brain barrier and into the CNS has posed a major obstacle for significant advances. We argue that it might not be necessary to overcome the blood-brain barrier but that brain endothelial cells may represent an excellent target for viral vectors to treat many neurological diseases. To explore this new concept we have investigated mouse models of Incontinentia pigmenti, a neurovascular disease affecting brain endothelial cells, and of Sandhoff disease, a lysosomal storage disorder. In both disorders, the transduction of brain endothelial cells by the intravenous injection of AAV-derived gene vectors brought considerable improvement of the clinical deficits. Based on these findings we suggest that gene vectors that target endothelial cells may be safe to administer and highly effective in some neurological disorders.

2.10 Biomimetic and stimuli-responsive biomaterials

Chairs: D. Fischer, G. Fuhrmann

SL.32

Nanoparticle-based Tissue Adhesives with Biomimetic Activity

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Wound complications remain a major cause of postoperative mortality. Recently, aqueous suspensions of metal oxide nanoparticles have been employed to connect biological tissue by serving as an adhesive layer. Here, I will present the prospects of this nano-bridging effect for tissue regeneration. Bioactive tissue adhesives composed of nanoparticles are produced via scalable and sterile flame spray pyrolysis. The process modularity allows adjustment of both particle composition and architecture and enables tailoring of the bioactivity. For example, catalase and sodium dismutase enzyme mimicry can be achieved by introducing ceria as nanozymes. We demonstrate highly modular synthesis of nanoparticles expressing adhesive properties in conjunction with tailored bioactivity. Such bioactive nanoparticles as adhesion nuclei in wound healing have a wide range of potential applications in surgical wound care and regenerative medicine.

We acknowledge support from the Bangerter-Rhyner Foundation and the Helmut Horten Foundation.

[1] Matter M.T. et. al., Nanoscale, 9, 2017.

SL.33

Nano Carriers and Structured Hydrogels containing Amino Acids

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Components and principles of the extracellular matrix are inspiring various approaches for the design of carriers systems and biomimetic materials. Here macromolecular architectures containing naturally occurring -amino acids are introduced, which were designed to exhibit multifunctionality. Polydepsipeptides, alternating copolymers of an α-amino acid and a α-hydroxy acid, are an interesting group of degradable polymers, and can exhibit strong physical interactions. Triblock copolymers with an oligodepsipeptide middle block build nanoparticles, which form stable polyplexes with great potential to combine efficient transfection capability with low toxicity of the transfection agent. [1] When incorporated in multiblock copolymers oligodepsipeptides are able to form stable hard domains with crystallites embedded in a glassy matrix. Such thermoplastic elastomers show very high elastic deformability and are capable of a reprogrammable shape-memory actuator function.[2] The requirements for biomaterials envisioned to induce an in vivo regeneration of tissue comprise complex combinations of different functions. A porous hydrogel with a biopolymer network architecture allows a modular approach for the creation of multifunctionality. Besides the molecular level, the three dimensional structure of the shaped body offers various options to implement functions associated to different hierarchical organization levels. These principles are illustrated by example of a cell-instructive gelatine-based 3D architectured hydrogel, which is able to modulate tissue regeneration in vivo.

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SL.34

Extracellular vesicles as biogenic carriers for therapeutic applications

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Extracellular vesicles (EVs) are natural lipid-based membranous particles decorated with surface and membrane proteins produced by almost any cell [1]. EVs are nature's way to deliver information as they transfer protein and nucleic acid based cargoes selectively to their target cell. Moreover, EVs feature a naturally derived composition; can potentially bypass complement activation and coagulation factors leading to reduced immunogenicity and increased stability in biological fluids [2]. These properties in conjugation with the potential ability to transfer information between cells locally and at distance have created excitement in the biomedical field [3]. Although initial clinical trials are ongoing, the use of EVs for therapeutic applications may be limited due to the lack of efficient loading methods for small molecules or their biotechnologically accessible upscale production. Moreover, potential "dilution effects" upon systemic administration or undesired off-target activity may affect their ability to reach their target tissues.

In this work, we discuss the potential of EVs from various cellular sources for drug delivery applications. We have recently developed different active methods for loading of model compounds into EVs and have shown that they deliver their information at the cellular level [4]. Furthermore, to tap the EVs' full therapeutic potential in a localised manner, we created a biomedical hydrogel containing EVs designed to achieve local delivery of therapeutics [5]. Due to the challenge of incorporating EVs into hydrogels without compromising their biological constitution, such approach had not been developed yet. To visualise EVs within hydrogels, we took advantage of sensitive density-dependent colour scanning electron microscopy [6], a powerful tool to spatially localised EVs within 3D-hydrogels. Taken together, we have created an important foundation for characterising EVs and understanding their cellular interactions which will be crucial for their advanced biomedical development.

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SL.35

Amphiphilic Nanogels: Tailoring Network Hydrophobicity to Dermal Delivery Applications

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The efficient dermal delivery of hydrophobic drugs requires new concepts in design and function of nanocarriers to address the following two main aspects: On one hand, the carrier properties have to account for the complex structural aspects of skin as challenging biobarrier. On the other hand, the delivery vehicles have to enable efficient hosting and release of drug molecules with limited solubility.

Polymeric nanogels have evolved as promising carriers to increase required drug compatibility due to their unique combination of colloidal size and internal network structure. However, up to now, the potential of nanogels for dermal delivery is limited due to their completely hydrophilic network structure. Such overall hydrophilic carriers are dramatically limited in their interaction with the amphiphatic environment of skin and are restricted to the delivery of drug molecules that are mostly hydrophilic. To overcome these limitations, we have developed a new type of polymer nanogels with precisely tunable amphiphilicity. These new nanocarriers combine multiple characteristics in a single colloidal system (Scheme 1): A hydrophilic polymeric matrix ensures biocompatibility and flexibility of the nanogels. Randomly distributed hydrophobic groups along this network will form hydrophobic domains within the nanogel to act as reservoir for the efficient encapsulation of hydrophobic drugs. In addition, the inherent amphiphilic nature of the nanogels, allows for dynamic reorganizations at the nanogel surface to adapt the carrier hydrophilicity to its surroundings. Depending on the carriers' location in the skin, these adaptive nanogels can present suitable groups at the particle surface, thus matching required amphiphilicity and enhancing skin-carrier interaction

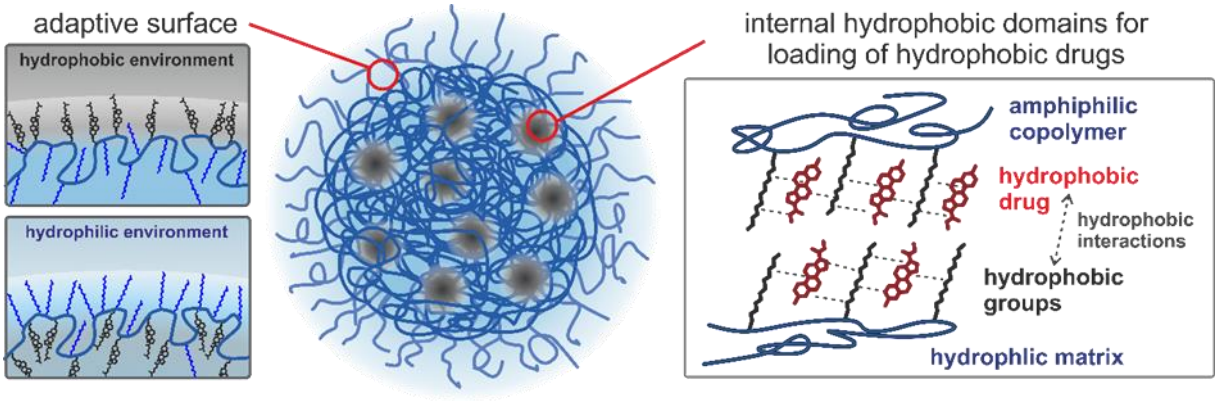

Figure1. Amphiphilic nanogels with adjustable network amphiphilicity to combine adaptive surface properties with tunable drug hosting and release in dermal delivery applications.

Preparation of such amphiphilic nanogels represents a significant synthetic challenge. Due to the dramatically different solubility of the two building blocks, existing emulsion-based approaches can show dramatic variations in the nanogels' colloidal features upon changing the hydrophilic/hydrophobic ratio. Since this hinders accurate development of structure-property relations, the carriers' colloidal features should remain unaltered upon changes in the hydrophobic domains to ensure comparability between different systems.

To address this challenge, we have developed a new synthetic method that is based on a master batch of crosslinked precursor nanogels, which serves as a platform for subsequent functionalization with hydrophilic and hydrophobic moieties. By this, de-coupling the interior network functionality from the colloidal properties of the particles can be achieved. Following this approach, we have prepared a small library of particles to systematically investigate the influence of nanogel amphiphilicity on drug loading capacity and the carrier interaction with excised human skin. It was shown that delivery of nile red as hydrophobic model compound to the viable epidermis was highest for nanogels with medium hydrophobicity, thus indicating the existence of an optimum carrier amphiphilicity. In combination with the good biocompatibility of these new carriers, these results suggest the high potential of the amphiphilic nanogels for mild yet efficient dermal delivery.

SL.36

Antitumoral potential of the myxobacterial peptolide Vioprolide A

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Vioprolide A, a cyclic depsipeptide obtained from the myxobacterium Cystobacter violaceus is described as cytotoxic agent. However, detailed studies concerning the biological profile, the mode of action and the molecular target(s) of the myxobacterial compound in cancer cells are not available yet. Hence, next to evaluating the anti-tumor potential of Vioprolide A in distinct cancer cell types, we aim to understand its underlying mechanism of action.

Interestingly, nanomolar concentrations of Vioprolide A inhibit the proliferative capacity of Jurkat ALL cells and reduces the growth of the bladder carcinoma cell line T24. Especially Jurkat cells reveal a high rate of apoptosis upon treatment with Vioprolide A, whereas the compound has no cytotoxic effect on healthy lymphocytes isolated from human blood. Boyden chamber assays as well as three dimensional spheroid migration assays revealed a strong anti-migratory effect of Vioprolide A treated T24 cells.

Concerning its mode of action, fist data indicate that Vioprolide A interferes with the translation machinery of cancer cells. Proteome data of Vioprolide A treated cancer cells demonstrate a significant diminished expression of the translation initiation factors eIF4A1 and eIF4H, which are known to be important for unwinding highly complex structures within the 5'UTR of RNA transcripts. Of note, mRNAs of several oncogenes like ADAM9, Myc and Mcl-1 consist of these secondary structures.

Therefore, we hypothesize that by inhibiting the expression of eIF4A1 and eIF4H, Vioprolide A impedes the translation of pivotal growth and survival factors which results in inhibition of tumor growth and metastasis.

SL.37

Stimulus sensitive HPMA polymers for tumour therapy: stimulus selection, optimisation and in vivo performance

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The use of polymer drug conjugates is a promising strategy to improve cancer therapy. In order to increase the specificity and to decrease the systemic toxicity, the polymer conjugate should accumulate in the tumour and release the drug locally by a specific stimulus. Tumour accumulation by passive targeting (EPR effect) is strongly size dependent [1] and requires long circulation times and the avoidance of the RES mediated uptake. Using multispectral optical Imaging [2], we followed the biofate of different carriers (polymer nanoparticles, nanocapsules, polymer-drug conjugates). In contrast to PEG-PLGA nanoparticles, HPMA polymers showed only very minor liver uptake and long circulation times. We explored the possibility to use different stimuli: e.g. cleavage by enzymes [3], redox mediated release [4] and pH-sensitive drug release [5]. Promising results were observed with all three strategies. Under consideration of additional aspects (e.g. polymers synthesis) we decided to focus on pH-sensitive systems. The linker has a key impact on the pharmacokinetics and tumour accumulation. A premature release will result in a fast excretion and poor accumulation. However, low efficacy will also be observed if the stimulus mediated release is to slow or does not occur at all. Therefore, we compared the impact of the linker on pH-meditated drug release *in vitro* and *in vivo* [6]. Finally, we used Doxorubicin-HPMA conjugates with pH-sensitive release to treat the resistant human germ cell tumour 1411HP in a preclinical model. Treatment with the pH-sensitive Doxorubicin-HPMA conjugates lead to high tumour accumulation and full regression of the tumour (Fig. 1), [7]. In addition, staining for hypoxic regions gave evidence for the correspondence of microacidic and hypoxic regions [7].

In conclusion, pH-sensitive HPMA drug conjugates are promising systems to improve tumour therapy.

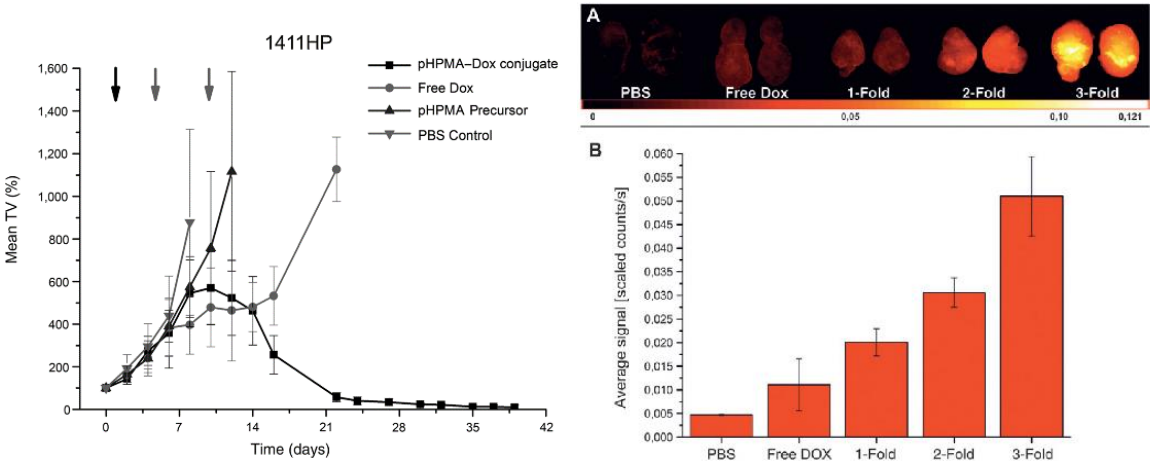

Figure 1: Left: impact of treatment on tumour volume of the doxorubicine resisent tumour 1411HP. Right: *Ex vivo*

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2.11 Mass Spectrometry

Chair: M. Lämmerhofer

SL.38

Proteome analysis by mass spectrometry

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For the analysis of proteomes mass spectrometry is the key technology, which meanwhile allows to identify and quantify thousands of proteins. The currently most often used approach for the analysis of a proteome is termed bottom-up proteomics, which is including the following steps. After obtaining a protein extract the proteins are digested, usually with trypsin, the resulting peptides are separated by liquid-chromatography (LC), transferred via an electrospray-ionization (ESI) source into a tandem mass spectrometer (MSMS), where the molecular masses of the peptide ions and their fragments are measured. The LC-MSMS data are processed and the resulting peak lists compared by a search engine with those of protein databases for the identification of the proteins. The relative quantification including statistics is performed by algorithms such as MaxQuant or Skyline. Two major types of quantification are established, label-based and label-free quantification [1]. The label-based approach is utilizing the integration of heavy isotopes into proteins (metabolic labelling: SILAC) or derivatization of tryptic peptides (e.g. with TMT). Quantification is performed by comparing the signal intensities in the mass spectrum of light and heavy peptide pairs, having the same sequence. The label-free approach is employing the area under the curves of extracted ion chromatograms of individual peptides for their quantification. Quantitative differential proteomics is well suited for identification of biomarkers – molecules which have key roles in defined molecular cell physiology or in the pathogenesis of diseases. Using the SILAC strategy e.g. we identified ANXA3 as a regulator of hepatitis C virus (HCV) maturation and egress [2]. Sample preparation is a critical step in every proteomic study. We demonstrated by differential proteomics via label-free quantification that sampling of tissues with a picosecond-infrared laser (PIRL) is giving better yields regarding the total amount and number of individual proteins [3]. Proteomics based on digestion of proteins is associated with the disadvantage that protein species, also termed proteoforms or isoforms, cannot be differentiated [4, 5]. For investigating protein species/proteoforms top-down mass spectrometry (TD-MS) was introduced [6]. In TD-MS of proteins, also termed top-down proteomics, intact proteins are transferred into the mass spectrometer, separated in the gas phase and fragmented. Especially for the analysis of protein species it is very important, that during homogenization the species are not converted by chemical or enzymatic reactions. We showed that also for this aim PIRL is well suited: Because of the very fast transfer of species from the intact tissue into the gas phase and the subsequent trapping of the proteins, both types of reactions are reduced to a minimum [7]. Bottom-up and top-down proteomics workflows both are applied for the comprehensive analysis of therapeutic proteins (TPs), because during the production of TPs a larger number of species always are generated. The problem is, that the action of some of these species is significantly reduced. Although occurring very seldom, species may even have severe side effects, e.g. as reported by Seidl et al. [8]. The authors showed that a species of erythropoietin, which is non-covalently binding tungsten ions, is forming aggregates, which can induce the generation of neutralizing antibodies in humans.

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SL.39

MS Binding Assays for the Characterization of Ligand Affinity and Drug Screening

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MS Binding Assays represent a label-free alternative to radioligand binding assays, providing basically the same capabilities as the latter.

Thereby, *MS Binding Assays* have the advantage that any ligand addressing the respective target with suitable affinity and potency may be selected as reporter ligand provided its quantification by MS allows a high enough sensitivity. Moreover, *MS Binding Assays* overcome all the drawbacks associated with radioisotopes as they rely on label free ligands as reporter ligands only, which are commonly termed MS Markers with reference to their purpose.

Alike radioligand binding assays, *MS Binding Assays* can be carried out in form of saturation experiments aiming at the determination of the K_D or as kinetic experiments to evaluate the rate constants of marker binding. Competition experiments finally allow to indirectly measure the affinity of test compounds by monitoring the binding of the MS Marker.

In the lecture, the use of *MS Binding Assays* for the characterization of binding affinities, for the performance of simultaneous binding assays, and in library screening will be highlighted [1].

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SL.40

Pitfalls of lipid mediator quantification in biological matrices using LC-MS/MS

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Quantification of lipid mediators in biological matrices is a very challenging procedure, which can be affected by several factors like instability of the analytes in the matrix or during the analytical process, availability of adequate standards and internal standards, limitations of the analytical technique, etc.

Even by using such a very selective and sensitive technique as mass spectrometry coupled to liquid chromatography most of the pitfalls of the quantification of lipid mediators in bioanalysis cannot be avoided. The presence of isomeric and particularly enantiomeric compounds, the low expected concentrations and potential instability of the analytes in the studied matrices as well as the lack of suitable internal standards are the most affecting factors in the reliable quantification of lipid mediators. These limitations of the analytical methods must be studied and control approaches beyond method validation for the developed LC-MS/MS methods should be included during sample analysis.

One example for a problematic bioanalysis of lipid mediators is the measurement of endocannabinoids in human plasma samples. There is a significant difference in the obtained concentrations depending on the additives used for sample collection and the storage time of the collected blood before and after centrifugation to obtain plasma samples. Another example for challenging bioanalysis is the determination of specialized proresolving lipid mediators. These compounds are light sensitive and adequate precautions have to be taken for the analysis of these analytes. This applies not only for the analytical but also for the preanalytical part of the analysis.

When the variables affecting the preanalytical conditions are controlled, variables affecting the analytical procedure take effect: many compounds experience isomerization, like 1- and 2-arachidonoyl glycerol. Furthermore, multiple interferences appear in the different biological matrices, for example when determining prostaglandins in mouse serum. The validation of the method is no guarantee for excluding such problems in the routine analysis. Particularly in the case of bioanalysis of endogenous compounds, since no blank matrices are available for the validation and normally alternative matrices are used. Therefore, it is of vital importance to establish further control mechanisms in addition to method validation, like, participation in interlaboratory comparisons and measurement of quality control samples and control samples in original matrices for each batch when conduction the bioanalysis of lipid mediators.

2.12 Antibacterial Natural Products

Chairs: C. Ducho, R. Holl

SL.41

Nucleoside antibiotics as antibacterial agents

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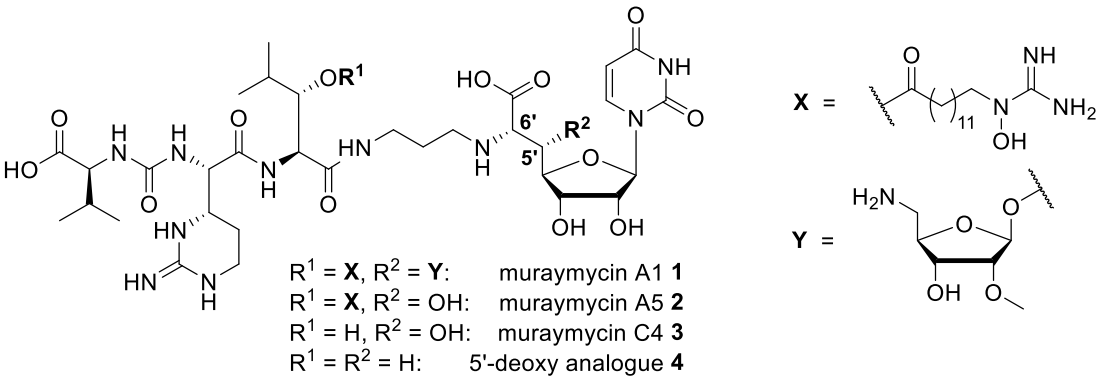
Several nucleoside analogues are highly valuable antiviral or anticancer drugs, but no antibacterial nucleosides are clinically established yet. At the same time, emerging resistances of bacterial strains towards established antibiotics cause an urgent need for the development of novel antibacterial agents. One approach to achieve this goal is the systematic investigation of naturally occurring antibiotics – including nucleoside derivatives – with new or previously unexploited modes of action.

Muraymycins (e.g. **1**-**3**, see Figure) represent a subclass of uridine-derived nucleoside antibiotics and were first isolated from *Streptomyces* sp. as a collection of 19 compounds [1]. They inhibit the bacterial membrane protein translocase I (MraY), a key enzyme in the intracellular part of peptidoglycan biosynthesis and therefore an attractive target for novel antibacterial drug candidates [2,3].



We have developed efficient synthetic methods for the preparation of muraymycins and their analogues, e.g. 5'-deoxy muraymycin C4 **4** (see Figure) [4-9]. Using a fluorescence-based *in vitro* assay for MraY activity [10], structure-activity relationship (SAR) data were obtained [11]. These results as well as further studies [12] on the properties of naturally occurring muraymycins and their synthetic analogues will be presented.

Acknowledgements: Deutsche Forschungsgemeinschaft (DFG, SFB 803 "Functionality controlled by organization in and between membranes" and grant DU 1095/5-1), Fonds der Chemischen Industrie (FCI, Sachkostenzuschuss) for funding.

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SL.42

Induction and quantification of drug uptake into Gram-negative bacteria

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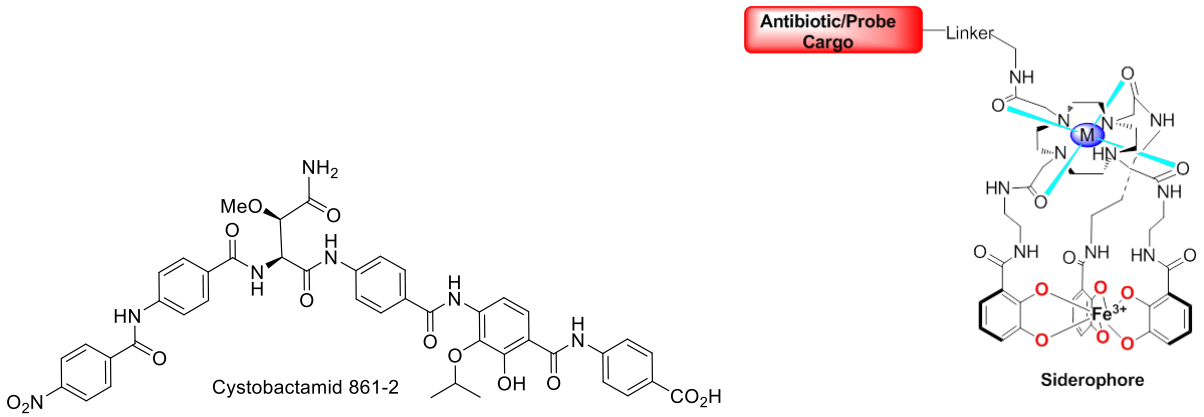
Infections caused by pathogenic bacteria represent a major health threat that is expected to rise further in the future. The need for novel antibiotics is currently not met by R&D efforts, in particular in the area of infections caused by Gram-negative bacteria. A main scientific hurdle is the lack of understanding how to assure a sufficient translocation of bioactive molecules across the Gram-negative cell wall.[1]

A lead finding strategy with proven track record focuses on natural products from microorganisms that have solved the penetration problem in an evolutionary process. The cystobactamids, gyrase inhibitors isolated from *Cystobacter* sp., represent a novel lead series with an unusual structure composed of PABA oligomers and potent, broad spectrum activity against Gram negative bacteria.[2,3] We will report three modular synthesis to cystobactamid analogs, and their application to generate (i) probes for cellular biology research and (ii) analogs with improved antibacterial properties and *in vivo* efficacy.

In an alternative lead finding strategy, our efforts to induce an active transport of small molecules into Gram negative bacteria will be presented. We report the design, synthesis and characterization of a series of theranostics agents based on 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid amide (DOTAM) derivatives,[4] comprising siderophores that are internalized into Gram negative bacteria, inhibit bacterial growth and demonstrate efficacy to visualize bacterial infections in mice by optical imaging in vivo. In addition, three orthogonal approaches (growth recovery, FAP, fractionation coupled to LC/MS/MS) to quantify the intracellular accumulation of such conjugates will be presented.



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SL.43

Synthesis and Chemical Biology Studies on Thiopeptide Antibiotics Structure and Activity

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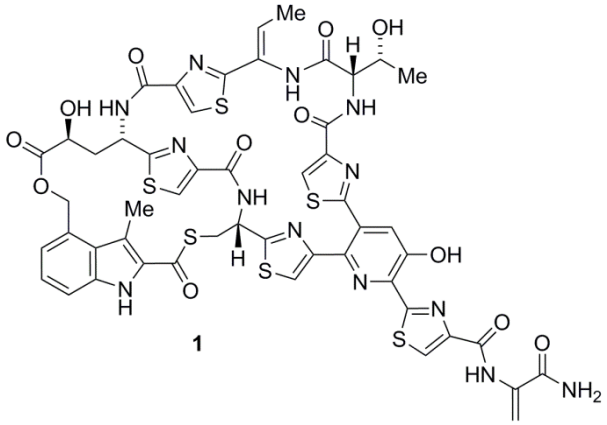
Thiopeptide antibiotics[1] are highly enticing natural products produced by ribosomal peptide synthesis.[2] Among them, nosiheptide (1) as a representative compound shows very high potency as an antibiotic and carries unusual structural features such as a 3-hydroxypyridine core and a thioester bond with in the B-ring macrocycle. We have completed the first successful total synthesis of this bismacrocyclic thiopeptide antibiotic by assembling a fully functionalized linear precursor and consecutive macrocyclizations. Key features were a critical macrothiolactonization and a mild deprotection strategy for the 3-hydroxypyridine core.[3] In this contribution highlights of the chemistry developed for this project will be presented, as well as its extension toward the synthesis of topologically different derivatives that are non-biogenic. Bioactivity of these compounds against pathogenic bacteria will be discussed, including *Staphylococcus aureus*, *Enterococcus faecium* and *Mycobacterium tuberculosis.* Furthermore, extensions of earlier PICC covalent reactivity studies[4] with the fully assembled 70S bacterial ribosomal will be presented that have led to a biochemically consistent binding mode hypothesis for nosiheptide at the primary target site.



1

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SL.44

The Hunt for Novel Antiinfective Compounds - Synthesis of Novel Rubrolide Analogues

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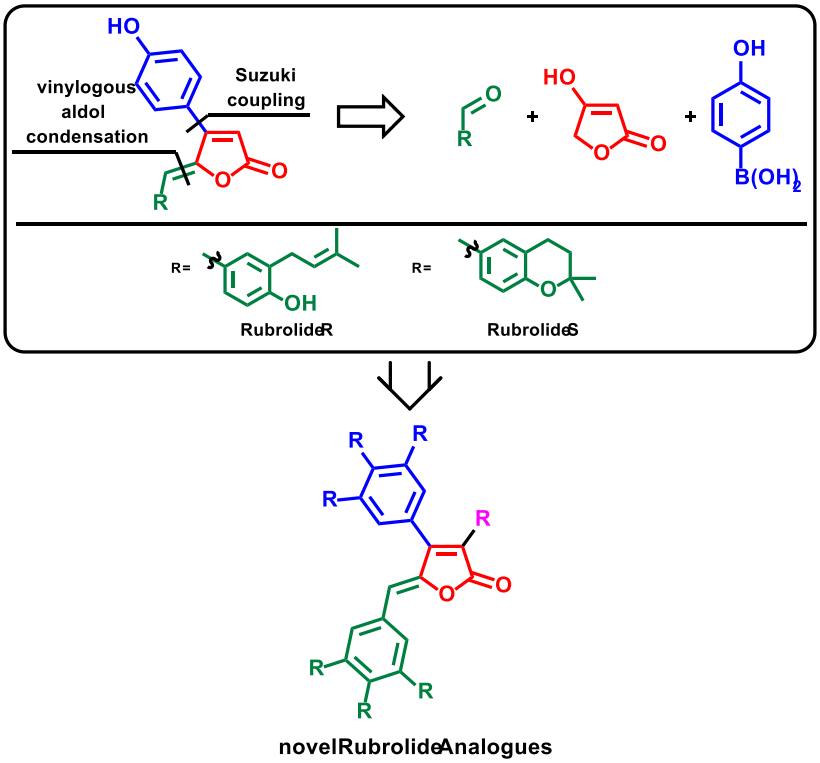
Due to the increasing resistances of bacteria and viruses against the established antiinfective drugs the treatment of infectious diseases is one of the challenges in drug development.[1] Natural products from plants, fungi, bacteria, marine organisms often led to novel drugs, which are today still important for the treatment of infectious diseases (e.g. erythromycin) or serve as lead structures for more potent analogues [2,3]. In most cases, promising natural products can only be isolated in small amounts from their natural sources limiting the screening for their potential biological activities. Therefore, the development of robust synthetic strategy accessing novel analogues is highly desirable.

An interesting example is the natural product class of the rubrolides. This marine-derived natural product family has an interesting portfolio of different biological activities [4]. In 2014, rubrolide R and S were isolated from the fungus Aspergillus terreus (OUCMDZ 1925), which was derived from barracuda intestines [5]. Both structures have been synthesized in a short and protecting group free sequence of three linear steps and were further evaluated in regard to their antiviral and antibiotic activities [6]. The natural products now serve as lead structures for analogues, which are further analyzed for their biological impact and their molecular target.



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2.13 From Structure to Function with Modern Techniques in Structural Biology

Chair: C. Betzel

SL.45

Serial crystallography at the European XFEL: First results and future opportunities

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Serial crystallography at X-ray Free Electron Lasers (XFELs)[1] has proven a valuable addition to the tool chest of structure determination techniques available today, particularly for time-resolved studies involving either photo-sensitive systems[2,3], radiation damage sensitive sample[4] or potentially for systems involving the mixing of substrate with a system under study[5]. The European X-ray Free Electron Laser[6] presents a new capability in performing serial crystallography experiments not just because additional XFEL sources create a higher availability of experimental time, but also because the European XFEL offers the highest repetition rate of XFEL pulses of all XFELs with orders more pulses per unit time.

In this presentation I will outline the experimental capabilities of the Single Particles, Clusters and Biomolecules and Serial Femtosecond Crystallography (SPB/SFX) instrument[7] of the European XFEL (EuXFEL), which is designed to predominantly support structural biology applications. I will show some select results from the very first experiments at the EuXFEL which demonstrate that we can successfully exploit the unprecedented repetition rate of EuXFEL for structural biology[8,9] and give insights into future experiments that may be performed at SPB/SFX.

Finally, if time, I will present the already planned expansion and upgrade of intrumentation at SPB/SFX. This includes the addition of a second interaction region allowing additional experiments in parallel or in differing configurations as well as further diagnostics to provide better and more complete metadata for analysis of XFEL diffraction data.

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SL.46

Serial Crystallography - Exciting possibilities for time resolved Structural Biology

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An overview will be given on recent developments in XFEL- and synchrotron based serial crystallography towards time-resolved structural biology. Methods will be presented and discussed, as well as limitations and future prospects to accurately unravel the structure and dynamics of biological systems with high temporal and spatial resolution.

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SL.47

Thermodynamic and Kinetic Elucidation of Protein-Ligand Interactions by Kinetic Isothermal Titration Calorimetry and Macromolecular Crystallography

Steffen Glöckner[1]; Andreas Heine[1]; Gerhard Klebe[1]

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The search for the underlying principles of protein-ligand binding has taken drug development from affinity-based via thermodynamically optimized compounds to the field of protein-ligand kinetics: At what rate do protein-ligand complexes form and disassemble? How can these rates be modulated? Are such properties directly correlated and can they be read off from a high-resolution crystal structure of the corresponding protein-ligand complex? And, above all, why are binding kinetic properties important for the design of novel medicines? While both thermodynamic and kinetic quantities of a protein-ligand binding event influence affinity, it is their individual characteristics that determine how a ligand's affinity for its target is conveyed and how this affects the biological system to which it is administered.[1,2] Thermodynamic optimization of a lead compound is a long-standing concept that has proved itself e.g. in the development of HIV-therapeutics.[3] With the introduction of the residence-time concept it became apparent that the time an inhibitor spends blocking its target is a quantity that can positively correlate with a drug's efficacy.[2] More recently it was also observed that an inhibitor's association rate can have beneficial impact on *in vivo* target occupancy or the reduction of side effects.[4,5] While the relevance of both the thermodynamics and kinetics governing a protein-lead system is accepted, their elucidation still relies on different techniques. Isothermal titration calorimetry (ITC) is the only technique that yields, apart from a ligand's affinity, the enthalpic and entropic contributions of the binding event.[6] Surface plasmon resonance (SPR) is the method of choice for obtaining kinetics.[7] The comparably new technology of kinITC enables extraction of kinetic data from calorimetric measurements and can therefor provide a full characterization of a protein-ligand system while adding value to resource-demanding ITC measurements. This talk will present examples of how kinITC and high-resolution macromolecular crystallography can be combined to simultaneously establish structure-thermodynamic and -kinetic relationships for a series of chemically minimally altered ligands and aims at conveying how ITC measurements can be optimized for time- and material-efficient extraction of biophysical data.

Acknowledgments: Malvern Panalytical, Helmholtz-Zentrum Berlin Macromolecular Crystallography Group

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2.14 Clinical Pharmacy to Improve Medication Safety

Chairs: C. Langebrake, M. J. Hug

SL.48

Population pharmacokinetics of midazolam in combination with fentanyl in critically ill children

Schultze-Florey, M.[1]; Würthwein, G.[2]; Hempel, G.[2]; Wicha, S.G.[3]; Blohm, M.[4]; Baehr, M.[1]; Langebrake, C.[1]

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Analgosedation represents a central therapeutic element of intensive care treatment to alleviate pain and prevent anxiety. Balancing the dose of analgesics and sedatives to achieve these therapeutic goals while avoiding adverse drug events and complications poses a major challenge.[1,2] Pharmacokinetics are often altered in both critically ill patients and children and despite the frequent use of midazolam and fentanyl for long-term analgosedation of paediatric intensive care patients, only limited data is available on pharmacokinetics in this population.[3]

In order to investigate the population pharmacokinetics of midazolam in combination with fentanyl in paediatric intensive care patients, a prospective, observational trial was conducted in the paediatric intensive care unit of the University Medical Centre Hamburg-Eppendorf. Patients receiving midazolam and fentanyl who were expected to be mechanically ventilated for at least 3 days were enrolled in the study. Blood samples for the determination of serum concentrations of midazolam were collected once daily and additionally during wash-out periods after discontinuation of midazolam infusion. Samples were analysed with liquid chromatography/mass spectrometry. The pharmacokinetic analysis was performed with nonlinear mixed effects regression methods using the software NONMEM® (version 7.4, ICON development solutions). One- and two-compartment models were evaluated. Different scaling models were tested on the pharmacokinetic parameters to account for changes in body weight and organ maturation. Covariate effects of bilirubin, ALAT, ASAT, INR, glomerular filtration rate, albumin, mortality score PELOD-2 and co-medication on pharmacokinetic parameters were tested. A potential interaction between midazolam and fentanyl due to CYP3A4 inhibition, as suggested by adult and *in vitro* data, was also considered.[4,5]

The study population comprised 21 patients aged 0.5 to 13 years, presenting a broad spectrum of diseases and organ insufficiencies. Midazolam pharmacokinetics was best described by a one-compartment model with a proportional and additive residual error model and interindividual variability on clearance and volume of distribution. Allometric scaling of body weight was added to the structural model parameters, but the data revealed no influence of maturation on midazolam clearance. Further significant covariates on midazolam clearance were bilirubin, as a parameter for liver function, and co-medication with fentanyl ($p < 0.01$), which considerably reduced midazolam clearance. In the final model, substantial interindividual and residual variability remained.

Despite the small patient sample size, patient heterogeneity and large variabilities which limit the model's predictive power, the results provide important evidence on covariates influencing the pharmacokinetics of midazolam. Taking into account those factors when dosing midazolam could contribute to improve medication safety in critically ill children.

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SL.49

Closed Loop Medication Administration – Medication Safety for Inpatients

Michael Baehr
University Medical Center Hamburg Eppendorf, Hospital Pharmacy

Medication errors represent one of the greatest risks for hospital patients. According to valid estimates, approximately 200,000 hospital admissions and 10,000 to 24,000 deaths are expected due to medication errors in Germany each year. Reasons for gaps in medication safety are increased medical complexity, outdated supply chains and paper-based processes. While discussions about "Digital Health", "Hospital 4.0" and the "Watson Era" in German hospitals did not begin until 2017, the University Medical Center Hamburg-Eppendorf (UKE) already started in 2008 to introduce a comprehensive electronic medical record and a closed loop of medication administration (CLMA) process. The main components of CLMA are a computerized physician order entry system (CPOE), clinical pharmacists who validate the orders at point of care, unit dose supply and electronic documentation of the administration.

According to a clear schedule, which is based on the ward round times, approx. 15,000 units are daily produced and delivered by the pharmacy. Oral medication is individually packaged and labelled with essential patient- and drug-related data such as patient's name, ward, room number, the drugs trade and free name, instructions for intake as well as date and time of administration. In addition, the entire leaflet is available via QR code at all times. Thus, the unit dose pouch has become a valuable vehicle of information. Likewise, all individually dosed, non-oral preparations, such as ampoules, injection bottles, pre-filled syringes, etc. are supplied patient-related.

To close the loop nurses register the administration to the electronic medical record. This ensures that all healthcare professionals have access to the current state of medication.

After ten years of experience, the digital and highly automated process is now established with high acceptance. In particular, nurses experience a great work relief as an error-prone process is delegated to the pharmacy. The effectiveness of the CLMA in terms of medication safety was investigated in a study (1) where a total of 3,111 medications were analyzed at two UKE wards. 95.6 percent of these medications have been delivered via the unit dose supply of the pharmacy and 4.4 percent – mainly PRN medication – have been given manually out of the ward stock. In total, 49 discrepancies were determined at both wards. This corresponds to a deviation rate of only 1.6 percent. According to an analogous study (2), the discrepancy rate is 56 percent in the traditional, paper-based and ward stock system. The results show that the paperless, digital CLMA is superior to the traditional, paper based ward stock process and leads to greater safety in patient care.

While the unit dose supply has so far only been possible for adults, since 2017 the pediatric clinic has been provided with the system. This presentation will report about current results.

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SL.50

The Role of Pharmacists in Antibiotic Stewardship Teams

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The rapid worldwide increase in antibiotic resistance is considered a threat to the therapy of infectious diseases and patient safety [1]. The relationship between antibiotic use and antibiotic resistance has been proven [2]. To combat the increase in resistance, reduction and optimization of antibiotic use are necessary. About 50 % of all in-patients are on antimicrobial therapy. Up to half of those antibiotic treatments are inappropriate or not necessary [3, 4]. For this reason, antibiotic stewardship (ABS) programs were and still are developed and implemented. They aim to *„continuously improve the quality of anti-infective prescribing […] in order to maximise clinical outcomes while minimising toxicity to the patient as well as the emergence of resistance and costs"* [5].

The german-austrian S3-Guideline "strategies to enhance rational use of antibiotics in hospital" [5] states two requirements, four core-strategies and four supplemental-strategies for the implementation of successful ABS programs in hospitals. As described in the ABS-requirements, the multidisciplinary stewardship team ought to include a clinical pharmacist, ideally with infectious diseases training. Some of the ABS strategies do need the pharmacist's general expertise on drug treatment. This includes - among other tasks - the development of local treatment guidelines, the compilation of an antibiotic drug formulary, restrictions of antibiotic use, elicitation of data on antimicrobial consumption and costs and giving suggestions concerning dose adaptation, drug administration or drug interactions. Several studies have shown the positive impact of pharmacists on the adoption and the results of ABS projects [6 – 11].
But still the implementation of ABS programs has to be improved. Recent surveys in different countries have investigated the current ABS activities and the involvement of hospital pharmacists [12 – 17]. The surveys indicate that the realization of ABS programs is also dependent on the legal framework of a state.
A current non-representative survey among 164 German hospital pharmacists reveals that only 65 % of these hospitals provide multidisciplinary ABS teams [17]. However, almost all of the surveyed hospitals have implemented at least one ABS activity. In 87 % pharmacists are participating in the implementation of ABS-core-strategies. While pharmacists are most frequently (74 %) involved in surveying the antibiotic use, authoring an antibiotic drug formulary, implementing approval requirements and performing therapeutic drug monitoring for selected antibiotics, the participation of pharmacists in on-site consultations (54 %) and in infectious disease consultation services (13 %) should be enhanced. Taking into consideration that the majority of the surveyed hospitals features pharmacists with completed or ongoing ABS-training courses (70 %) and/or advanced training in infectiology, further reinforcements of pharmacist-driven ABS-projects are expected.

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SL.51

"AMTS, ARMIN, ATHINA": Clinical pharmacy in the ambulatory care setting of Germany – an update on the current path

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Even though German community pharmacists in 2018 are highly trained and respected health care specialists who do work for the benefit of their patients in about 20000 community pharmacies that provide unlimited and easy access for the patients, the provision of medication analysis and management is not yet standardised nationwide.

In 2012 Förster and Pfeifer published an analysis of the status of pharmaceutical care practice in Germany. Related thereto is this critical update of new pharmaceutical services offered in German community pharmacies in 2018 and the alternatives offered by other players in the healthcare community.

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2.15 Epigenetics

Chairs: M. Jung, F. Hansen

SL.52

Chemical tools to advance epigenetic biology and drug discovery

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A plethora of studies have demonstrated the critical importance of epigenetic mechanisms, i.e. posttranslational chromatin modifications to control phenotypic hallmarks in a wide range of human diseases, raising the possibility to develop novel epigenetic-based therapeutic concepts in e.g. oncology, inflammatory or degenerative diseases. Despite the significant progress in developing molecular genetic tools to study epigenetic mechanisms, the availability of well-characterised chemical tools to study epigenetic biology and validate novel targets is comparably low. To overcome this impasse, the Structural Genomics Consortium, an international public-private partnership consisting of academic and private partner networks engaged in target discovery, develops novel chemical tools for 'pioneer' i.e. unexplored target areas including 'readers, writers and erasers' of a histone or chromatin code.

This presentation will give an overview on progress to date and illustrate possible achievements and pitfalls of selected molecules to understand epigenetic cancer biology and advance target validation.

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SL.53

Target discovery beyond the BET-Family: novel chemical probes for less studied bromodomains

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With the first inhibitors of the Bromodomain and Extra-Terminal domain (BET) subfamily, a remarkable success story of Bromodomains as novel drug targets has been started. However, first evaluations of ongoing clinical trials revealed that treatment with BET-inhibitors can be accompanied with significant toxic side effects and the therapeutic benefit of BET-inhibitors still remains to be validated [1]. Non-BET bromodomains such as those in p300, CBP or BRPF1 are less studied. Here we show that 4-acyl pyrroles can be applied to develop potent inhibitors not only for the BET-family but also for several other bromodomains. One of these inhibitors, XDM-CBP, is highly selective for the bromodomains of CBP and p300 and was derived from a BET-BRD-binding fragment. After optimization based on structural modeling, X-ray crystal-structure analysis and thermodynamic profiling, XDM-CBP was used in screenings of several cancer cell lines *in vitro* to study its inhibitory potential on cancer cell proliferation. XDM-CBP is demonstrated to act on specific cancer cell lines, in particular on malignant melanoma, breast cancer, and leukemia [2].

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SL.54

Structure-based design of selective HDAC8 inhibitors

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Histone deacetylases are validated drug targets for the treatment of cancer; so far six HDAC inhibitors have been approved for clinical use in T-cell lymphomas and multiple myeloma. Besides their use as anticancer drugs, HDAC inhibitors show promising potential for the treatment of other human diseases such as parasitic infections.[1]

The presented work focuses on the development of selective HDAC8 inhibitors to explore their utility in the treatment of schistosomiasis and neuroblastoma. In the course of this study, we were able to successfully apply structure-based techniques to identify and optimize compounds as potent and selective inhibitors of schistosomal and human HDAC8.

Several studies have established an important role of HDACs in the life cycle of Schistosoma; where Schistosoma mansoni HDAC8 (SmHDAC8) was found to exhibit the highest abundance in all stages of the parasite‘s lifecycle.[2,3] The application of virtual screening campaigns combined with in vitro testing guided us to the identification of several small molecule inhibitors of SmHDAC8.[4,5] Several of the identified hits were successfully co-crystallized with SmHDAC8 [3], paving the way for structure-based optimization studies. This led to the development of several benzhydroxamate derivatives showing high inhibitory activity against SmHDAC8, selectivity against major human HDAC isoforms and in vitro antischistosomal activity.[6]

With some of the identified benzhydroxamate derivatives showing high activity against human HDAC8, the compounds were further optimized to obtain potent and selective HDAC8 inhibitors with anti-neuroblastoma activity.[7]

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SL.55

Development of RTS-V5 as the first-in-class dual histone deacetylase-proteasome inhibitor

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The approach “one drug multiple targets” or “multi-target drugs” is gaining major consideration in drug discovery and has been termed polypharmacology.[1] Despite the highly significant therapeutic relevance of combination therapies, potential advantages of a targeted therapy based on a single drug acting through two or more independent modes of action include (a) a more predictable pharmacokinetic profile, (b) increased patient compliance, and (c) the simultaneous presence of the molecule in tissues where the active principle is needed to work.[1] Proteasome inhibitors possess synergistic activity with histone deacetylase (HDAC) inhibitors due to the simultaneous blockage of the proteasome and aggresome pathway leading to accumulation of misfolded proteins. Here, we present the rational design, synthesis, binding modes and anticancer properties of RTS-V5 as the first-in-class dual HDAC-proteasome inhibitor.

RTS-V5 was designed by hybridizing the pharmacophores of the HDAC6 selective HDACi of type **I** [2] and the non-covalent proteasome inhibitor compound **ML16** [3] (Figure 1). Subsequently, we synthesized RTS-V5 from known building blocks via a multi-step synthetic protocol. The inhibition of both targets was confirmed by biochemical and cellular assays as well as X-ray crystal structures of the 20S proteasome and HDAC6 complexed with RTS-V5. Cytotoxicity assays with leukemia and multiple myeloma cell lines as well as patient-derived leukemia cells demonstrated that RTS-V5 possesses potent and selective anticancer activity. Furthermore, we show that RTS-V5 induces apoptosis and blocks proliferation, cell cycle, colony formation and aggresome accumulation. Taken together, we have discovered RTS-V5 as the first-in-class non-covalent dual HDAC-proteasome inhibitor.

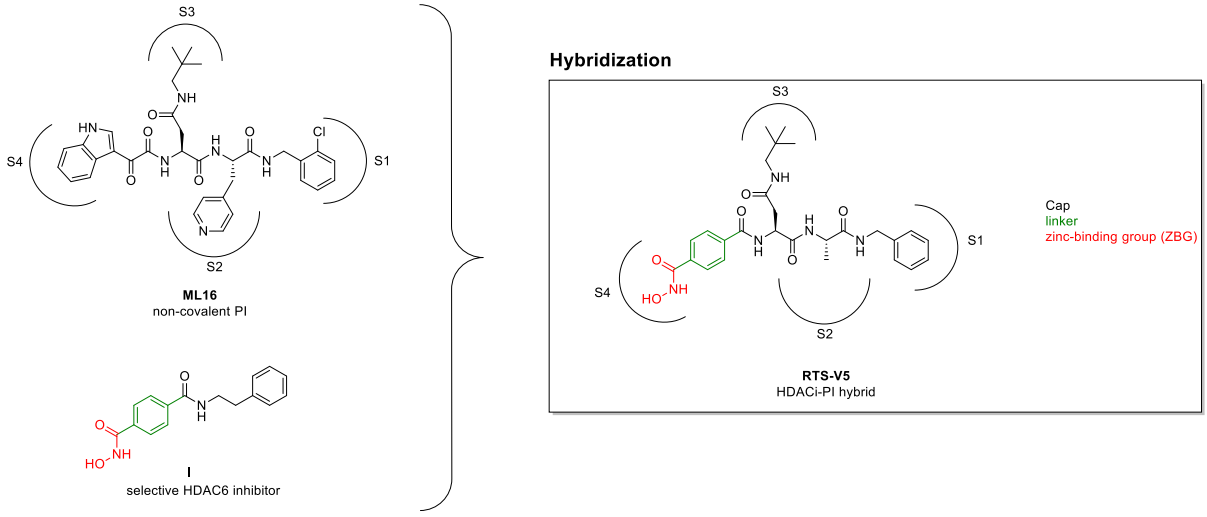

Figure 1. Design of the first-in-class dual proteasome-HDAC inhibitor **RTS-V5**.

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SL.56

Cofactor-based Chemical Probes for Lysine Acetyltransferases

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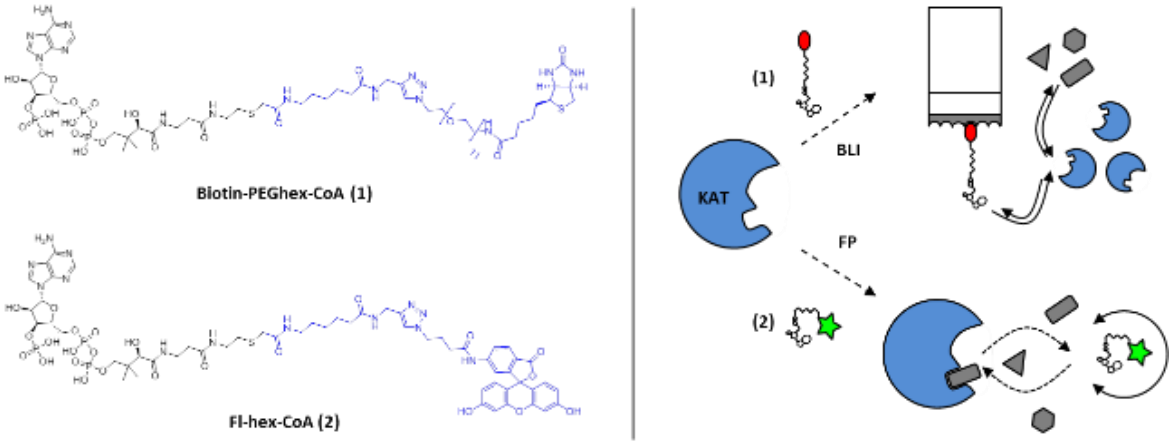
Lysine acetyltransferases (KATs) are a group of enzymes that catalyze the acetylation of substrate lysine residues by employing the cofactor acetyl-CoA. Together with other epigenetic posttranslational modifications (e.g. methylation, ubiquitinylation, sumoylation, ADP ribosylation), acetylation serves as a regulating tool to control protein function.[1] Because of the deep implication of KATs in cell development and their frequent aberration in different malignancies, small molecule modulators of KAT activity are urgently needed to assess their therapeutic potential and for probing their underlying biology. Although KAT enzymes can be grouped into distinct families based on their sequence similarity and catalytic mechanism, their mode of action is characterized by a strong initial interaction with the cofactor followed by a rather weak binding of the lysine substrate. Because of this difference in substrate/cofactor interaction and affinity, the development and optimization of assays and the identification of small molecule inhibitors has proven to be challenging.[2] The concept of using CoA-derived inhibitors and probes has mainly been used for the identification and characterization of new enzymes and substrates.[3] Here we present the design and synthesis of two functional cofactor-based chemical probes and their application as mechanistic tools in a broadly applicable assay platform. In this platform, a fluorescence polarization (FP) based binding assay is combined with biolayer interferometry (BLI) competition analysis to enable easy, reliable, and profound screening for ligands that target the KAT cofactor binding site.



Acknowledgments: Deutsche Forschungsgemeinschaft (DFG SPP 1463 and RTG 1976), Dr. Tobias Rumpf and Dr. Asifa Akhtar (MPI Freiburg)

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2.16 Protein Kinase Inhibitors - Novel Inhibition Mechanisms

Chairs: S. Laufer, C. Kunick

SL.57

Halogen bonding as concept for protein kinase inhibitor design

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While the complex $I_2 \cdots NH_3$ was reported in the middle of the 19th century as product of the reaction between ammonia and iodide [1], the general ability of the heavier halogens (Cl, Br, I) to form non-covalent bonds with Lewis bases was recognized in the 1950s [2]. It took another six decades until in 2013 the IUPAC published an official definition of the term "halogen bond" for the phenomenon [3]. In recent years this kind of supramolecular interaction has raised considerable interest in medicinal chemistry. It was realized that numerous halogenated bioactive compounds interact with their biological targets via halogen bonding [4]. Namely for a number of protein kinases inhibitors halogen bonds were identified as relevant for alignment within the ATP binding pocket [5,6]. In many of these cases, the halogen atom is attached to an aromatic ring of the inhibitor and interacts with a backbone carbonyl oxygen of the hinge area [6,7,8]. However, other Lewis-basic positions of the host kinase may be also addressed by heavier halogen atoms, e.g. aromatic rings and other π-systems [9,10] as well as chalcogen atoms within amino acid side chains [11]. It has been a question of debate for some times whether water molecules may act as halogen bond acceptors, a fact that is now generally accepted [12]. The introduction of halogen substituents into distinct positions of a protein kinase inhibitor scaffold has repeatedly been suggested as a method for the structure-based rational design of inhibitors [4,5,7]. However, it becomes more and more obvious that tailoring halogen bonds is not a trivial task, because added halogen atoms strongly influence the molecular properties of the parent molecule on the one hand and halogen bonds are sterically challenging on the other hand. The talk will present examples from the literature for different kinds of halogen bonding between inhibitors and their protein kinase targets, and will highlight a case in which a distinct water molecule is addressed within the ATP binding pocket [13,14].

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SL.58

Targeting the R-Spine: Design, Synthesis and Biological Evaluation of Novel Type I½ p38α MAP Kinase Inhibitors with Excellent Selectivity, High Potency and Prolonged Target Residence Time: Implication for Cancer- and CNS-Applications.

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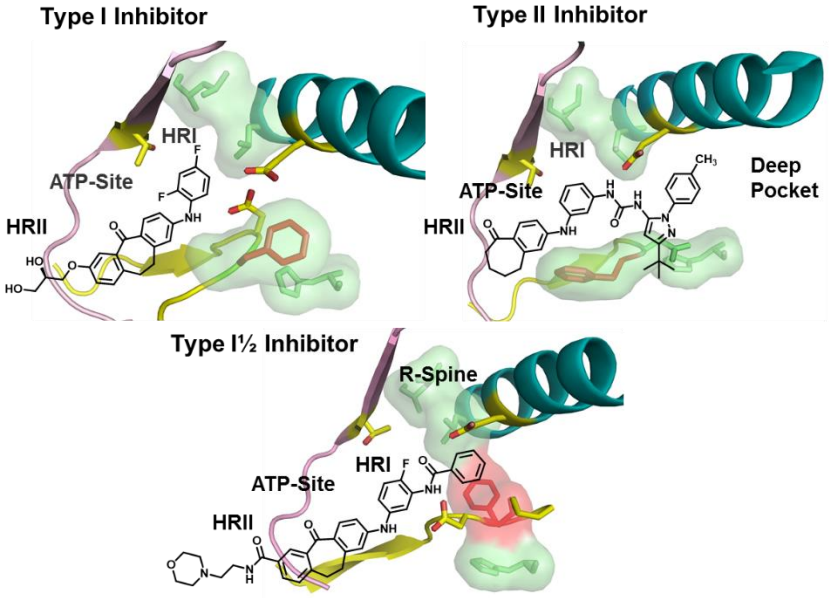
p38 MAP Kinase inhibitors are widely investigated for a plethora of inflammatory diseases including RA and COPD. Latest results however may open an avenue for cancer and CNS-diseases as well. For such indications however, Inhibitors with very particular properties are necessary.



We recently reported Skepinone-L as a Type I p38α MAP kinase inhibitor with high potency and excellent selectivity *in vitro* and *in vivo*.[1] However, as a Type I inhibitor it acts entirely ATP competitive and shows just a moderate residence time. Thus, the scope was to develop a new class of advanced compounds maintaining the structural binding features of Skepinone-L scaffold like inducing a glycine flip at the hinge region and occupying both hydrophobic regions I and II. Extending this scaffold with suitable residues resulted in an interference with the kinase's R-Spine. By optimizing this interaction, we could significantly prolong the target residence time up to 4.000 s, along with an excellent selectivity-score of 0.006 and an outstanding subnanomolar potency. This new binding mode was validated by cocrystallization, showing all binding interactions typifying Type I½ binding.[2,3]

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SL.59

Investigating Cellular Mechanism-of-Action and Target Engagement with Chemical Biology

K. Huber

Drug target identification and validation represent paramount objectives for chemical biology. Selective small molecule tool compounds and chemical probes are highly complementary to genetic target validation approaches and can facilitate drug development. Conversely, the cellular phenotype of many clinical candidates and even approved drugs is often driven by polypharmacology, i.e. the modulation of several cellular targets in parallel. This phenomenon can occur within a given target class, e.g. kinases, but can also affect unforeseen targets outside a protein family. Recent advances in chemical biology have provided a suite of tools to further elucidate the cellular effects of compounds in cells and patient tissue which in turn can support lead optimisation and even patient selection.

SL.60

Reversible and irreversible inhibitors of c-Jun N-terminal kinase 3

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The cJun N-terminal kinases (JNKs) belong to the family of mitogen-activated protein (MAP) kinases and are activated by different forms of cellular stress. They catalyze e.g. the N-terminal phosphorylation of the transcription factor c-Jun. There exist three different types of JNKs, which are encoded by different genes, namely JNK1, JNK2 and JNK3.[1] Whereas JNK1 and JNK2 are present in all cells and tissues of the human body, JNK3 is almost exclusively expressed in the brain. The deregulation of this JNK isoform is often linked with neurological disorders, like Parkinson's and Alzheimer's disease.[2,3]

Starting from known pyridinylimidazole-based p38α MAP kinase inhibitors, reversible and irreversible inhibitors of the JNK3 were synthesized. Altering the substitution pattern of the pyridinylimidazole scaffold proved to be effective in shifting the inhibitory activity from the original target to the closely related JNK3. In particular, a significant improvement for JNK3 selectivity could be achieved by addressing the hydrophobic region I with a small methyl group. The most potent inhibitor of the reversible series inhibits the JNK3 in the low triple-digit nanomolar range, is metabolically stable and displays a slight selectivity over the JNK2 isoform.[4] The binding mode at the enzyme's ATP binding site for this class of compounds was confirmed by X-ray structures of JNK3 crystals incubated with different reversible inhibitors.

In a second strategy, the concept of covalent inhibition of c-Jun N-terminal kinase 3 (JNK3) was successfully transferred to our pyridinylimidazole scaffolds varying several structural features in order to deduce crucial structure–activity relationships.[5] Joint targeting of the hydrophobic region I and methylation of imidazole-N1 position increased the activity and reduced the numbers of off-targets. The most promising irreversible inhibitor inhibits the JNK3 in the subnanomolar range shows high metabolic stability in human liver microsomes and displays excellent selectivity in a screening against 410 kinases. Covalent binding to Cys154 of the JNK3 was confirmed by incubation of the inhibitors with wild-type JNK3 and a JNK3-C154A mutant followed by mass spectrometry.

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SL.61

Blocking Traffic Flow – Cdk5 Inhibition Introduces Novel Ways to Prevent Sorafenib Treatment Escape in HCC Cells

Maximilian A. Ardelt[1,2], Thomas Fröhlich[3], Emanuele Martini[4], Martin Müller[1], Veronika Kanitz[5], Carina Atzberger[1], Petra Cantonati[2], Martina Meßner[1,2], Laura Posselt[6], Thorsten Lehr[7], Jan-Georg Wojtyniak[7], Melanie Ulrich[1], Georg J. Arnold[3], Lars König[6], Dario Parazzoli[4], Stefan Zahler[1], Simon Rothenfußer[6], Doris Mayr[5], Alexander Gerbes[8], Giorgio Scita[4], Angelika M. Vollmar[1], Johanna Pachmayr[1,2]

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6 Center of Integrated Protein Science Munich (CIPS-M) and Division of Clinical Pharmacology, Department of Internal Medicine IV, Klinikum der Universität München.

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Introduction: Therapeutic options for advanced-stage hepatocellular carcinoma (HCC) patients are very limited. Sorafenib which still represents the only approved first line therapy shows low response rates and severe side effects.[1] Compensatory activation of growth factor receptors upon Sorafenib accounts for treatment escape.[2] However, approaches to combine Sorafenib with specific inhibitors of individual growth factor receptors have failed.[3] Here, we investigated cyclin dependent kinase 5 (Cdk5) inhibition as a promising combination strategy to improve Sorafenib response in HCC.

Methods: Functional effects of the combination of Sorafenib and Cdk5 inhibition were evaluated by applying genetic knockdown (siRNA/shRNA, CRISPR/Cas9) and clinically tested inhibitors of Cdk5. The mode of action of Cdk5 inhibition to improve Sorafenib response was judged by a LC-MS/MS-based proteomic approach. Clinical relevance was proved by a human HCC tissue microarray. Intracellular receptor trafficking was investigated by confocal microscopy with live cell imaging and sophisticated analysis of vesicle properties.

Results: Combination of Cdk5 inhibition and Sorafenib synergistically impaired HCC progression in vitro and in vivo, by inhibiting both tumor cell proliferation and migration. We could confirm that HCC cells use an upregulation of the EGFR signalling cascade to evade Sorafenib treatment. Through the inhibition of Cdk5 the compensatory activation of growth factor receptors could be prevented. Of note, the observed effects are regulated by a novel mode of action for Cdk5: Cdk5 inhibition interferes with intracellular trafficking which is crucial for growth factor receptor signalling, thereby leading to enlarged endosomal vesicles and accumulation of respective cargo proteins. In contrast to the unsuccessful specific inhibition of individual growth factor receptors, Cdk5 inhibition offers a comprehensive approach to block the compensatory activation of growth factor receptors upon Sorafenib in general.

Conclusion: Cdk5 inhibition represents an effective approach to improve Sorafenib response and prevent Sorafenib treatment escape. As Dinaciclib is a clinically evaluated and well-tolerated Cdk5 inhibitor, our study provides evidence for clinically evaluating the combination of Sorafenib and Dinaciclib to improve the therapeutic situation for advanced-stage HCC patients.

Acknowledgement: Financial support by DFG (VO376/17-1) is gratefully acknowledged.

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2.17 Drug delivery of poorly water soluble molecules - from salt design to carriers

Chairs: L. Meinel, H. Bunjes

SL.62

From Molecule to Molecular Dispersion – Enabling Formulations via Hot-Melt Extrusion

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Formation of amorphous solid dispersions (ASDs) is a powerful formulation principle to enhance bioavailability for poorly soluble drugs (BCS II and IV).

During dissolution of the polymeric system, the molecularly dispersed drug is released, usually resulting in a supersaturated solution of enhanced bioavailability. Next to spray drying (SD) hot-melt extrusion (HME) is the main manufacturing process for ASDs avoiding the search of a common solvent for drug and polymer however, at the cost of higher thermal exposure and potential degradation.

Starting point is the selection of the embedding polymer, which later on governs the site of dissolution and the extent of supersaturation and kinetics [1]. Next to theoretical/simulation approaches, analytical characterization by DSC e.g. melting point depression, determination of dissolution endpoint, or monitoring of glass transition temperatures (Tg) are common tools to assess the solubility of drugs within the polymeric matrices. The application of a complex mathematical model (BCKV-equation) to describe the dependency of Tg on the active pharmaceutical ingredient (API)/polymer ratio enables the prediction of API solubility at ambient conditions (25 °C). Furthermore, estimation of the minimal processing temperature for forming ASDs during HME trials could be defined [2]. In a next step, the drug/polymer combination of choice needs to be processed in an extruder. Process conditions (throughput, screw speed, temperature profile and screw profile) can be estimated with the help of process simulation, which also enables the assessment of mostly adiabatic process conditions for a later scale up [3]. Among the input variables for process simulation, melt viscosity is most crucial and rather laborious to measure. However, quick estimates of melt rheology via a correlation with Tg are available [4].

Performance characterization of ASDs and ranking with other formulation approaches can reliably determined using non-sink dissolution methods with pH-shift and an optional organic absorption sink compartment [5].

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SL.63

Rational design and characterization of lipid based DDS for oral administration of poorly soluble drugs.

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Lipid based drug delivery systems (LBDDS) are one of the key technologies to overcome the problems of poorly soluble drugs. LBDDS should preferentially retain the drug in the solubilized state and provide a high drug absorption with minimized food dependency. LBDDS cover a broad range of different properties and structures. The classification systems developed by Pouton [1] is used worldwide as a tool for a rational development of LBDDS. After oral intake, LBDDS will undergo processes of dilution and digestion. Both processes might change the solubilisation capacity of the LBDDS for the incorporated drug. The dilution factor and the microenvironment covers a broad range of variability. Protocols have been developed to mimic the *in vivo* behaviour *in vitro* in order to select the most promising LBDDS for clinical studies. They involve the dilution in different environments and include also the impact of enzymatic digestion processes (e.g. by pancreatin). In most cases, the pH-stat method is used to detect the lipase induced formation of fatty acids. However, recent studies show that this method might lead to wrong results due to incomplete titration (20-100%) due to the strong variability end environmental dependency of the apparent pKa values of fatty acids [2]. Therefore, alternative methods (e.g. HPTLC, HPLC) should be considered. Recent studies also show, that many surfactants are partially digested and lose their stabilizing activity. The digestibility is quite diverse for different surfactants and impacts the performance of the LBDDS. Non-digestible surfactants might also impact the degree of lipase induced digestion. For example, a pancreatic lipase specific digestion of polymeric nanoparticles (can be blocked by orlistat) has been observed for Poloxamer and Tween stabilised PLGA-nanoparticles, but not for PVA-stabilised nanoparticles [3]. In many cases, middle chain glycerides have increased drug solubilities, but also show higher tendencies for digestion induced drug precipitation. This can be explained by the higher aqueous solubility of the digestion products and their decreased capability to form liquid crystalline phases. An observable trend in the development or oral LBDDS are formulations which avoid soft gelatin capsules and which can be processed by standard solid dosage technologies (pelletisation, tabletting). Both extrusion / spheronisation [4, 5] and adsorption on silicates [6] transforms liquid lipids into processable solids. However, the solid material might impact the performance by incomplete and selective release of different components of the LBDDS. Detailed information on the solidification process, the microenvironment and the existence of different immobilised species can be obtained by NMR, ESR and fluorescence techniques. In conclusion, both biological and biophysical aspects have to be considered to develop a robust LBDDS with good performance.

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SL.64

Steering the interplay of supramolecular species (aggregates, micelles, vesicles) in the gastrointestinal tract – a new paradigm in enabling formulations development

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The wetting of solid drug substances (DS) and/or the solubilisation of lipophilic DS in the duodenum/proximal intestine are critically impacted by bile acids and phospholipids [1]. This impact is the result of a complex interplay of various parameters which hitherto were rarely characterized let alone understood. Although this interplay is expected to be compound specific, generally applicable development processes are sought for the future, leading to blueprints for early pharmaceutical development. For example, fluctuation in pharmacokinetic outcome will become predictable while the necessary hypothesis-driven tools are at the same time identified steering critical DS into reliable drug products (DP).

In a case study we detailed for Imatinib, a BCS class II drug with high inter-subject variability in t_{max} [2, 3], solubilisation in simulated intestinal fluids containing taurocholate and lecithin by NMR spectroscopy, X-ray diffractometry, transmission electron microscopy, and dynamic light scattering analysis [4, 5]. In brief, Imatinib strongly interacted with taurocholate and to a lesser extent with lecithin in simulated fasted state (FaSSIF). Whereas large vesicles were formed at lower Imatinib concentrations within which the Imatinib was engulfed in their cores (≤ 250 µM; diameter >110 nm), exceeding this threshold resulted in a collapse to smaller micelles (< 40 nm) with the Imatinib still being in proximity to taurocholate and driven into the micelles' shell. In contrast, higher TC/L concentrations simulating fed state (FeSSIF) effectively controlled the micelle structure and molecular interaction of Imatinib throughout the entire DS concentration range.

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2.18 Supercritical Fluid Chromatography

Chair: M. Parr

SL.65

Beyond the Current State-of-the-Art in Pharmaceutical Analysis using Supercritical Fluid Chromatography (SFC)

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Supercritical Fluid Chromatography (SFC) has slowly become an important tool in pharmaceutical analysis over the last 20 years and has been adopted by most larger pharmaceutical companies, although it remains a relatively minor separation technique. Two of the primary applications have been chiral analysis (and chiral method development) and purification at the mini- and semi-preparative scale (up to Kg), where it is vastly superior to HPLC. On the same column, SFC is 3 to 5 times faster with 1/3rd to 1/5th the pressure drop of HPLC. However, the commercial SFC instrumentation available today is not on a par in performance with current ultra high performance liquid chromatographs (UHPLC's), with system variance more than an order of magnitude higher. As a result, the migration to sub-2µm, and small superficially porous particles has been slow in SFC.

Recent improvements in instrumentation, combined with relatively simple modifications have been shown to allow full theoretical efficiency with both achiral and chiral columns, with sub-2µm particles. Very fast, high efficiency analyses are possible and will be demonstrated. The robustness is now similar the HPLC. In addition, the understanding of the physical chemistry of the mobile phases has advanced over the last few years, since it is now possible to model CO2/MeOH densities and viscosities, along with issues such as Joule-Thompson heating vs. cooling (inaccurately called resistive heating in HPLC). These advances will be outlined. Some differences between SFC and HPLC that apparently have not been well understood will be pointed out.

Some future variations with regard to atmospheric pressure mini-prep separation and collection of minor contaminants and breakdown products, and chiral purification will be briefly discussed.

SL.66

Application of SFC(MS) in Drug Research and Development

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SFC has been successfully used within the Medicinal Chemistry Department at Boehringer Ingelheim in Biberach for several years. Especially the advantages in terms of speed and efficiency and savings in solvent costs led to the fact, that the majority of chiral separations at Boehringer Ingelheim are presently performed on SFC systems.

Important technical improvements have been developed and commercialized for analytical SFC and SFC-MS instruments in the last years. These developments have led to more reliable instrumentation. In addition, the orthogonality of SFC methods compared to HPLC, has led to a broader distribution of SFC within our company. SFC and SFC-MS systems are now used in the Chemical Development Department within a regulated environment.

In the presentation new analytical instrumentation within the Medicinal Chemistry Department and the qualification of SFC in Chemical Development will be presented.

Despite the improvement on the analytical site, little or less improvement has been achieved in the field of mass directed fraction collection using SFC-MS. In the presentation a new option for the transformation of an existing preparative LCMS into a preparative SFC-MS will be presented.

SL.67

SFC-MS in bioanalysis

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Separations by means of reversed phase (RP) HPLC and GC are the standard chromatographic methods in drug analysis and bioanalysis today. GC often provides higher separation efficiency, even than UHPLC, but lacks the possibility to analyse ionic or highly polar compounds. Especially the direct analysis of intact drug conjugates is impossible. RP-HPLC on the other hand shows limited resolution capabilities and highly polar analytes interact only insufficiently on the conventional analytical columns. Even on the more polar RP columns like phenyl-hexyl as well as HILIC columns their analysis remains challenging. A further challenging task in bioanalysis is the separation of enantiomeric compounds.

Supercritical fluid chromatography (SFC) gained importance in the last couple of years since new and robust instruments entered the market. Known to be a valuable option for the separation of enantiomers, SFC may also be an alternative to conventional HPLC and GC methods. The orthogonal chromatographic behaviour of SFC compared to HPLC as well as the possibilities to run at high flow rates yielding fast separations may drive SFC towards an even more significant chromatographic alternative.

Exemplary separations of drugs and their metabolites utilizing SFC coupled with tandem mass spectrometric detection are presented. Analytes over a broad polarity range as well as separations of isomers and chiral drugs are covered.

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3 POSTERS

3.1 Other Topics

POS.1

The journal publications by Rudolf Schmitz (1918–1992). An overview.

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The poster presents a view on the journal publications by Rudolf Schmitz, the first full professor of the history of pharmacy at the Philipps-University Marburg. The scientific work by Rudolf Schmitz is one aspect in our research titled „Rudolf Schmitz (1918–1992) and his scientific school in Marburg" which is to be examined besides other aspects. These aspects are the headmaster, the pupils, the style of work as well as the scientific and social appreciation.
This presentation only focuses on the journal publications by Rudolf Schmitz. At the same time, we contemplate the journals of various scientific disciplines and the published essays by him.
Starting with the first publications referring to Rudolf Schmitz' dissertation of the pharmaceutical chemistry in the journal „Chemische Berichte", the poster furthermore allows us to take a statistical view onto the journals in which Rudolf Schmitz most frequently published his essays. In addition to this, the journals are listed up, structured and assigned to different disciplines, for example chemistry, pharmacy, history of pharmacy as well as medicine.
Finally the poster gives us an insight into the various topics of Rudolf Schmitz' essays with the help of copies in the lower part of the poster.

POS.2

"Seit wann ist die Pharmacie eine Wissenschaft?" Doctoral degrees reflecting science genesis in the 19th century

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Obtaining a doctoral degree has been a milestone in the pharmaceutical development from craftsmanship to science at the end of the 18th as well as in the 19th century.
Pharmacists doing research were given the opportunity to receive a PhD at the University of Erlangen. Approximately until 1860, the University of Erlangen was a centre of doctoral degrees in absence: Beside a handwritten or already printed treatise, one characteristic at the Philosophical Faculty of Erlangen was answering questions arising from subjects belonging to the faculty in written form from the distance.
Among them, there were also many theories currently under debate, e. g. the question since when pharmacy was recognized as a science.
From the middle of the 18th century on, this topic was considered also for chemistry and mathematics.
This presentation sheds a light on the tasks of three PhD students at the Philosophical Faculty of Erlangen:
Carl Ludwig Hopff (1801–1849) was confronted with the question by professor Carl Wilhelm Böttiger (1790–1862) in 1825, whether pharmacy was a modern or rather old science. In his answer, he described the historical dimension of the work of pharmacists in great detail. At the same time, Christian Heinrich had to decide whether pharmacy was a true science and in which way it fitted into the major field of sciences. Gottlieb Reuss (born in 1811) asked himself in 1834, whether pharmacy was of scientific relevance. His answer included the definition of Emanuel Kant's way of observing the general concept of science. Reuss was moreover convinced that chemistry was the scientific component within the area of pharmacy.
The final conclusion of Hopff and Reuss was unanimous: Rational analysis of pharmacy started in the 17th century and continued until the 19th century. Antoine Lavoisier's (1743–1794) new system proved to be the major step forward towards the development of pharmacy.
Karl Wilhelm Gottlob Kastner (1783–1857), who had been a tenured professor for Chemistry and Physics in Erlangen during 1821–1857, was especially keen on advocating the concerns of pharmacists. Therefore, it was his main idea to introduce pharmacists as craftsmen by and by into science. He was the editor of scientific publications of pharmacists and supported them in order to receive their PhD at the faculty.
The wish to improve pharmacy as a scientific subject led to the fact of more pharmacists than ever before entering the field of research, especially in the 19th century.

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POS.3

Impact of cold atmospheric plasma on the melanogenesis in human melanoma cell lines

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The color of the human skin derives from melanocytes which are localized in the stratum basale of the epidermis. Formation and transport of melanin pigments is a well-orchestrated process in which various enzymes are involved. The activity of enzymes in the melanin formation is dependent on multiple factors, such as reactive oxygen species [1]. Cold atmospheric plasma is known to provide reactive nitrogen and oxygen species when applied to liquids, biological materials or tissue [2]. The impact of cold atmospheric plasma on the melanogenesis has not been investigated until now. This study uses different melanoma cell lines as a model to investigate the modification of melanin synthesis by cold atmospheric plasma-treated liquids. In preliminary experiments, the melanin content was determined by measuring the absorbance of sodium hydroxide-lyzed cells. In all tested cell lines, the melanin content increased after incubation with plasma-treated cell culture medium. Because of poor solubility of eumelanin in alkaline solution this method may lack the changes in eumelanin content but detects the increased amount of pheomelanin only. To include the eumelanin in the analytic approach, alkaline hydrogen peroxide oxidation [3] was performed and degraded products were separated by HPLC.
Furthermore, the impact of cold atmospheric plasma was tested in regards of morphology, using immunofluorescence, viability, using the resazurin assay and expression levels of microphthalmia-associated transcription factor (MiTF). Expression levels of MiTF have been examined before and after indirect plasma treatment by qPCR and will add to further understanding of the signaling cascade. In all tested cell lines, no changes in morphology were detected and the viability remained at least 80% compared with untreated control cells. Furthermore, the results obtained point towards an activation of the MiTF and therefore to an involvement of MiTF in plasma-activated melanogenesis.

This work was founded by the Ministry of Education, Science, and Culture of the State of Mecklenburg-Western Pomerania (Germany), the European Union, European Social Fund (grant numbers AU 11 038; ESF/IV-BM-B35-0010/13 and AU 15 001).

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POS.4

Autodisplay of Nanobodies

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Nanobodies are recombinant expressed variable domains derived from camelid single domain antibodies. The CDR3 of nanobodies forms long finger like extensions which are able to occupy cavities, as for example the active site of enzymes [1]. Combined with other advantages, in particular small size, high solubility, thermal stability, refolding capacity and good tissue penetration [2,3,4], nanobodies are interesting affinity molecules for therapeutic options as well as for diagnostic purposes.

In this proof of concept study, three different nanobodies were presented on the surface of *Escherichia coli* using an improved version of Autodisplay [5]. A first nanobody was directed against human epidermal growth factor receptor 2 (HER2), the second nanobody against the toxic microalgae *Alexandrium minutum* and the third nanobody was directed against mammalian extracellular vesicles. All three nanobodies were expressed alone or together with intracellular GFP. Surface presentation was proven by a proteinase K accessibility test in combination with SDS-PAGE. The co-expression of GFP was investigated using flow cytometry. To proof the functionality of the nanobodies on the cell surface binding assays have been performed.

The presentation of nanobodies on the surface of *E. coli* can be used manifold. The generation of libraries based on nanobodies can enable the identification of new variants. Furthermore, these nanobody-presenting bacteria offer many diagnostic opportunities. They can be used as a "biochromatographic substrate" or for the detection of an immobilized antigen enabled through the intracellular GFP fluorescence.

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POS.5

Appraisal of immunocapture by different antibodies prior to an surrogate peptide approach utilizing LC-HRMS analysis for quantification of the low abundant protein renin in human plasma

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Introduction: Protein quantification involves arduous method development with complex and time-intensive workflows. Although traditionally applied, ligand binding assays have several limitations in protein quantification because of their e.g. limited linear dynamic range, risk of cross reactivity across metabolites/analogs, and maintain difficulty with multiplexing. Novel LC-MS approaches have emerged as highly effective methods in protein bioanalysis and enabled low abundant proteins detectable in endogenous levels. However, the molecular weight of the target structure is a limited parameter in mass spectrometric (MS) quantification. The surrogate peptide approach for protein quantification utilizing trypsin for digestion of high molecular proteins is often used but makes digested biological matrix even more complex. A promising approach is to capture the target protein in biological matrix with antibodies prior to tryptic digestion. The immunocapture procedure needs a high affinity antibody which binds effectively and leads to a robust method. In this study, available antibodies were investigated concerning their usefulness for protein quantification of renin by MS. Renin was selected as its low endogenous levels makes LC-MS detection of tryptic peptides degenerated by trypsin in unpurified plasma challenging.

Materials and Methods: A BLAST® in silico experiment was used to identify a unique peptide of renin and the transitions for quantification by Shimadzu Nexera LC-system coupled with Sciex TripleTOF 6600 mass spectrometer. Human recombinant renin (Cayman chemicals) was spiked into 500 µL plasma per experiment. Four different monoclonal human renin antibodies supplied by R&D systems, SantaCruz biotechnology and DRG were evaluated on their affinity and binding efficiency. The incubation periods varied between 1 hour, 2 hours or overnight at 4 °C and were conducted either directly with plasma or crosslinked to magnetic dynabeads coupled with Protein G (ThermoFisher). The immunocapture was followed by tailored denaturation step with RapiGest, Urea and ammonium bicarbonate buffer as well as digestion with 200 ng Trypsin. For final clean-up, a custom-made µelution solid-phase extraction protocol was used.

Results: The BLAST experiment classified one peptide of renin (VVFDTGSSNVWVPSSK) as unique. LC-HRMS settings were successfully optimized to detect the transition m/z 854.9 to 418.2296. The developed immunocapture approach outmatched the classical digestion approach with regard to recovery and sensitivity (1.29E+03 cps vs. no detectable surrogate peptide). The direct comparison of approach A (renin captured in plasma and then eluted from beads bonded antibodies) vs approach B (digestion of beads bonded antibodies plus captured renin captured in plasma) for 1 h was similar for the R&D antibody (1.29E+03 cps for antibody bonded antigen vs 1.24E+03 cps for the antigen eluting approach). No substantial effect of the investigated incubation periods were observed for the antibodies captured renin (1.24E+03 cps for 1h incubation vs 1.44E+03 cps in overnight approach). The assessment of antibody affinity showed that R&D and DRG detection antibodies showed similar performance, while SantaCruz affinity was the lowest. The DRG catching antibody has up to 10x times more binding affinity which also improves the recovery of the method. The DRG catching antibody is also characterized by low interference with tryptic peptides after digestion. The different binding abilities may be explained by the different binding sites of the antibodies since the two DRG antibodies bind specifically to the active center but at different position. R&D antibody and SantaCruz antibody bind outside the active center which might lead to lower affinity to renin. Moreover, the study observed no difference in renin recovery if the elution of the antigen was performed with or without elution from magnetic beads prior to digestion.
Conclusion: The study highlighted the relevance of sophisticated investigations of available renin antibodies for the immunocapture approach and identified the most suitable antibody for further surrogate peptide method development. The method facilitates the reliable detection of renin in endogenous human matrix by LC-HRMS.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602295 (LENA).

POS.6

Ethnopharmacology in the Himalayas – From the records of the ethnologist René Mario de Nebesky-Wojkowitz (1923 – 1959)

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While investigating botanical explorers of the 18th, 19th and 20th century who were engaged in ethnobotany and ethnopharmacology, the archive material of the ethnologist Mr René Mario de Nebesky-Wojkowitz attracted attention. Although not linked to a natural scientific or a healthcare profession, his work is of valuable pharmaceutical-historical interest. The notes found [1, 2] contain information about the traditional

use of Tibetan and Nepalese medicinal plants, dating back to his extensive journeys in the Himalayas and its surroundings in the 1950s. Besides the indigenous names, their habitat and their traditional medicinal use by the natives were recorded. In addition, a correspondence between Mr de Nebesky-Wojkowitz and the Smith, Kline & French Laboratories was found, stating that he forwarded approximately 300 plants for further analytical screening which resulted in discovering alkaloid content and microbiological activity within certain plants. The aim of the work is the attempt to extract information from the historical records and compare them to evidence-based modern-day research [3]. Some of these findings might give rise to new leads and proposals for current phytopharmacological research.

This research was kindly funded by a stipend from the Hermann-Schelenz-Institute for the History of Pharmacy and Culture in Heidelberg e. V..

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POS.7

Cork – scarce commodity during the First World War in Germany

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Cork was an important and indispensable tool in the pharmacy and served above all to close the bottle of vessels. Due to its special properties (elasticity, low specific weight, good and easy processing) it could not simply be replaced. In 1913 approximately 10,000 tons of cork were imported, mainly from Spain and Portugal. Due to export bans of former partner countries as a result of the outbreak of the First World War, cork also suffered considerable Shortages.

As early as 1916, the Apotheker-Zeitung reads the following quote: "Den vielen gering geachteten Gegenständen, die der Krieg uns hat schätzen gelehrt, muß auch der leicht befundene Flaschenkorken sich zugesellen". Thus, in September 1917 cork wood, cork waste and semi-finished and finished products made from cork were confiscated by the Ministry of War and an inventory survey was introduced.

Also, there had been fixed maximum prices. Overall, in wartime, pharmacists industry suffered from a significant shortage of cork supply. For example, in the months of May/June 1918, only 75% of the applied demand from the pharmacies was covered through the “war raw materials department” and the pharmacies were requested to use substitutes for cork stoppers.

The replacement plugs were divided into two groups: a larger group comprising wooden plugs and a smaller group comprising plugs made of other materials. Pharmacists developed considerable creativity, as shown in the following example: In 1917, a pharmacist from Plauen and his friend working as a physician, reported an inedible sponge (Polyporus pinicola and marginatus), which could be found in the native forests in large numbers, as a substitute for cork. and received a patent for this as a rubber substitute. This so-called "Alsa-Cork" was popular among pharmacists for special applications.

Dr. H. Freund writes in the Pharmazeutische Zeitung in 1918: "Since the middle of 1917, over 100 different processes for the production of a replacement stopper have been patented in Germany. Already this amount proves how difficult it is to find a real replacement for the cork stopper...".

References:
Literature is available from the author on request

POS.8

The carbazole derivative C81 impairs angiogenic key features in endothelial cells *in vitro*

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Angiogenesis, the formation of new blood vessels from pre-existing capillaries, is a process that plays an important role in the normal physiology and during wound healing but also in pathophysiological conditions such as cancer or inflammation. Ongoing angiogenesis occurs in chronic inflammatory diseases such as psoriasis or rheumatoid arthritis amplifying the inflammatory response, finally leading to severe tissue damage. Moreover, in cancer, tumor growth and metastasis are strongly dependent on the formation of new blood vessels. Hence, inhibitors of angiogenesis are of great benefit as they might reduce inflammatory processes or prevent cancer cells from spreading and, therefore, help to shift from a pathological situation to the normal physiological status. As current anti-angiogenic pharmacotherapy is not always satisfying, there is a great need for the discovery of new drug leads. The small molecule C81 is a synthetic carbazole derivative and acts as a kinase inhibitor addressing many kinases such as BMP2K, AAK1 or CLK1/4 (determined by thermal shift assay) whose functions are, so far, widely unknown in the vascular endothelium. In this *in vitro* study we focused on the effects of C81 on angiogenesis-related processes using human endothelial cells. In initial experiments we showed that only very high concentrations of C81 compromise the viability of human umbilical vein endothelial cells (HUVECs) after 24 or 48 hours of treatment (IC_{50}: 24 h: 171 µM; 48 h: 51 µM). In *in vitro* assays addressing the key features of angiogenesis we demonstrated that C81 inhibits the proliferation of HUVECs with an IC_{50} of 7 µM. In addition, the undirected migration of endothelial cells (ECs) was strongly inhibited after C81 treatment (scratch assay, 10 µM). Besides the undirected migration we analyzed the effects of C81 on the directed migration of HUVECs towards a chemoattractant gradient. Here, C81 impaired the migration of HUVECs in the direction of a fetal calf serum (FCS) stimulus in a Boyden chamber assay significantly. In a second approach we performed 2D chemotaxis assays monitoring the migratory behavior of HUVECs in the direction of an FCS gradient after C81 treatment. In accordance with the results of the Boyden chamber assay, C81 strongly attenuates the chemotactic orientation of ECs indicated by a markedly reduced migration directness towards the chemotactic stimulus. In *in vitro* tube formation assays using Matrigel® we showed that C81 inhibits the formation of capillary-like structures in a concentration-dependent manner. Interestingly, C81 significantly impaired the VEGF-induced sprouting from HUVEC spheroids indicated by a reduced cumulative sprout length and mean number of sprouts, whereas the bFGF-induced formation of sprouts remained unaffected. Analyzing the ERK signaling pathway that underlies the process of angiogenesis by western blot, we found that C81 significantly reduced the VEGF-induced ERK activation while the bFGF-triggered ERK phosphorylation was not inhibited.
Our study provides first insights into the anti-angiogenic potential of the carbazole derivative C81 *in vitro*. The precise VEGF-specific mechanism being affected by C81 in endothelial cells during their angiogenic differentiation process is still unknown but will further be elucidated. Nevertheless, C81 represents a promising tool for the inhibition of angiogenesis and might be of high importance for various pathophysiological conditions.

POS.9

Initial-Answer Changing Behavior in Pharmacy MCQ State Exams

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The individual course of a written objective test with respect to the exam candidates' answering and answer-changing behavior has been evaluated various times within the past decades. A belief-in-intuition vs. logics/empiricism strategy in "one-best-answer" MCQ tests has been subject of discussions in a variety of academic fields. Instructors' recommendations to change or not to change the initial answer in academic written tests appear as popular wisdom rather than evidence-based (ref.). To provide an insight into this aspect, an evaluation of the four written tests that represent the first part of the German pharmaceutical state examination is hypothesized. These tests include pharmacy-related natural science skills, competencies and the application of these abilities.
Methods: In the current study, initial-answer changing behavior was investigated in a population of 1130 candidates and 360 MCQ test items in all four written exams altogether (=300860 answered items in total) in the nationwide and simultaneous Spring 2018 MCQ pharmacy state exams (specifications as given in the AAppO: all items were one-best-answer questions with five options, i.e., one answer out of five is correct, and the time frames of the exams with 90 sec per item are tight). For the purpose of this study, all 3344 IMPP answer sheets were -repetitively- electronically scanned to detect erased markings. The data were analyzed with respect to candidate variables (e.g., academic abilities) as well as item variables (e.g., p-values as measure of the levels of difficulty or item formats).
Results: The complete number of initial-answer changes amount to 1592 out of the 300860 (0.53 %). The majority of changes represent wrong-to-right (W→R) changes with 44.97 %, whereas 31.72 % were W→W and 23.30 % R→W changes. The wrong-to-right and right-to-wrong changes might affect the candidates final test scores.
The corrections are evenly distributed over the four different exams, which implies that there is no accumulation in one particular field. The occurrence of changes correlates significantly with the discriminatory power of items ($\propto < 5\%$) as well as the item-specific p-values, while no influence of the respective MCQ item format was detected.
Conclusions: These findings indicate, that the characteristics that are specific to natural sciences (such as the unambiguousness of the item content as well as the preciseness of phrasing) are crucial factors for the low number of initial-answer changing behavior, also with respect to initial answering, and would not support spontaneous, intuitive and potentially non-reflected answering strategies, which may be followed by initial-answer changing.

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Johnson, D., Anderson, S., Johnson, S.: *The United States Army Medical Department Journal* **2014**: 86-88

POS.10

Approved and non-approved selective absences in nationwide pharmaceutical MCQ state exams: Do subpopulation characteristics diverge from average candidate characteristics?

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The first part (P1) out of three parts of the German Pharmaceutical State Examination consists of a sequence of four independent MCQ exams (P1/I-IV; according to the German AAppO). Each of these written exams is simultaneous, nationwide, and semiannual, i.e., P1 is held twice per year. Due to the broadness and depth of the respective four disciplines / subject groups, candidates need to show continuous performance and persistence on four subsequent days.

Data: Since 2006, i.e., following the transition to the current composition of P1/I-IV (that includes, e.g., human biology on Day 2 and pharmaceutics on Day 3), approximately 21 K candidates were *newly* registered for P1, took the exams, and are subject to the current retrospective study aiming at detailed analyses of the group characteristics of selective non-attenders (appr. 10 %) for the whole 4-day period as well as for the single exam days: Day 1 "Chemistry", Day 2 "Pharmaceutical and human biology", Day 3 "Physics, physical chemistry, drug formulation", Day 4 "Drug analysis".

Results: A higher percentage of male vs. female candidates passes all four P1 exams upon their first trial. A higher proportion of Germans vs. non-Germans passes P1 upon the first trial.

Selective absences are highest on Day 2 -and this finding holds true for approved and non-approved absences as well as cumulative absences- followed by Day 3. In general, for the studied candidate data, final P1 exam scores are better for candidates with non-approved vs. approved absence.

Looking at gender aspects, average exam scores are slightly better for male than for female students, the relative frequency of approved as well as non-approved absences is higher for females vs. males.

Considering the subpopulation of students with foreign nationalities vs. German candidates, non-Germans show higher absence frequencies, which are twice as high as those of Germans for unapproved absence and three times as high for approved absence (sick note). Germans reach better P1-scores than non-Germans.

Moreover, the number of terms necessary to finish the first segment of pharmaceutical training and pass P1/I-IV tends to be higher for female than male students and higher for non-German than for German students.

Conclusions:

- Higher absence rates in the middle of the P1 exam period may be explained by the complexity of the exam content and the overall exam schedule.
- There appears to be a tendency for better P1 scores with shorter time (i.e., a smaller number of terms), irrespective of the reasons for delay.

POS.11

Behrings Tetanusserum Entwicklung, Herstellung und Einsatz

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Im Dezember 1890 veröffentlichte Emil von Behring (1854—1917) gemeinsam mit Shibasaburo Kitasato (1853—1931) den Aufsatz „Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren" in der Zeitschrift *Deutsche Medicinischen Wochenschrift*. In ihrer Publikation zeigten sie erstmalig, dass es ihnen gelungen war, an Tetanus und Diphtherie erkrankte Mäuse und Kaninchen zu heilen und gesunde Tiere gegen diese Infektionen zu immunisieren.

Nach vielen weiteren Studien hatte Behring es geschafft, ein am Menschen applizierbares und wirksames Tetanusserum zu entwickeln.

1903 schloss er mit der Marburger Firma *Dr. Siebert und Dr. Ziegenbein oHG* einen Vertrag unter anderem über die Produktion und den Vertrieb seines Tetanus-Heilserums. Ein Jahr später gründete er

zusammen mit den Apothekern Carl Siebert (1863—1931) und Hans Ziegenbein (1867—1920) die Firma *Behringwerk Marburg/Lahn*.

Das Behringwerk nutzte vor allem Pferde zur Produktion von Tetanus-Antikörpern durch allmählich gesteigerte Tetanusgift-Injektionen.

Ausgerechnet der Erste Weltkrieg brachte dem Unternehmen erste deutliche wirtschaftliche Erfolge durch den erhöhten Bedarf und Einsatz von Behrings Tetanus-Heilserum durch die deutsche Armee. Da das Tetanus-Antitoxin viele Soldaten vor der lebensbedrohlichen Krankheit bewahrte, würdigte man Behring mit dem Ehrentitel „Retter der Soldaten".

POS.12

Der Einfluss von Jöns Jacob Berzelius auf die Pharmazie und Chemie in Deutschland

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Jöns Jacob Berzelius (1779–1848) führte nicht nur die heutige Symbolschreibweise der Elemente ein, er galt zu seiner Zeit als „Papst der Chemie" und stellte neben Joseph Louis Gay-Lussac (1778–1850) den Lehrmeister neuer Chemiker dar. Viele seiner Schüler stammten aus Deutschland und konnten dank der Ausbildung bei Berzelius zu geachteten Wissenschaftlern aufsteigen. Viele Korrespondenzen mit ehemaligen Schülern wie Friedrich Wöhler (1800–1882) oder Gustav Magnus (1802–1870) sind bereits lange gedruckt erschienen, doch nun konnten die noch unveröffentlichten Schriftwechsel zwischen ihm und den Brüdern Rose, Söhne von Valentin Rose dem Jüngeren (1762–1807) und Schüler von Martin Heinrich Klaproth (1743–1817), ausfindig gemacht werden. Wie sehr der Einfluss von Berzelius auf seiner Schüler Heinrich (1795–1864) und Gustav Rose (1798–1873) auch noch nach deren Ausbildung war, steht im Forschungsmittelpunkt.

Kungliga Vetenskaps-Akademien, Centrum för Vetenskapshistoria, Anne Miche de Malleray

Hjelt, E.: Aus Jac. Berzelius' und Gustav Magnus' Briefwechsel in den Jahren 1828-184 (Verlag von Friedrich Vieweg und Sohn), **1900**.
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POS.13

Historical research from the British colonies: A grass from Africa as a repellent

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Back in 1921 the British botanist Morley Thomas Dawe (1880 – 1943) sent a memorandum to his employer, the Royal Botanic Gardens in Kew, London, reporting his observations on a strong-smelling grass, growing in Portuguese Congo (today's Province of Cabinda, Angola), known as "Efwatakala" and later identified as *Melinis minutiflora* P. Beauv.. It was traditionally used by the natives for preparing beds for their animals to prevent ticks and fleas sucking blood from them and also for driving away white ants by applying the grass to their nests. In addition, he noted that the grass is preferred by cattle and provides an excellent pasture for fattening them because of the oil yielded. After observing this effect on ticks and insects, Mr Dawe concluded that the grass might be a break-through in preventing further outbreaks of sleeping sickness in humans by controlling or even eradicating the disease-carrying Tsetse in certain areas of British West Africa. His plan was to cut down humid woodland, the natural habitat of certain Tsetse species which affected humans and regrow these areas with Efwatakala afterwards. Dawe's idea was considered worthwhile for further research by the Kew officials, prompting the procurement of larger amounts of seeds, distribution of them in the colonies, cultivation of plants and attempts to analyse the active repellent principle. Unfortunately, no evidence-based repellent effect of the grass was reported in the Kew correspondence until 1925. During the course of the 20th century the quality of the grass for pasture and more important as a tick repellent was reported in several agricultural publications. A systematic bibliographic analysis with the focus on German and English publications to evaluate the active compounds and the value of the grass as a repellent against the castor bean tick (*Ixodes ricinus* L.) which is feeding on humans is currently in process.

This research was kindly funded by a stipend from the Hermann-Schelenz-Institute for the History of Pharmacy and Culture in Heidelberg e. V..

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POS.14

Selective imaging of αvβ8-integrin with Ga-68-labelled peptide conjugates by means of positron emission tomography

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Integrins are a class of 24 known transmembrane receptors which mediate the adhesion of cells to the extracellular matrix, and form α-β heterodimers comprising one out of 18 α- and 8 β-proteins. Eight of them recognize the Arg-Gly-Asp (RGD) amino acid sequence, among them the well-known αvβ3 integrin. However, the less well-known αvβ8-integrin is the least studied among the 8 RGD-binding integrins because no selective small-molecule ligands for have been available. αvβ8-integrin features a unique stucture in that it does not possess a cytoplasmic tail. It plays a key role for fundamental processes of tumor cell development and differentiation, for example, activation of TGFβ1, and is involved in tumor progression and invasion. Furthermore, it mediates the cellular entry of certain viruses, such as Foot-and-Mouth (FMDV) Herpes Simplex (HSV) or Epstein-Barr (EBV). We present the first small-molecule ligand for αvβ8-integrin, and the corresponding ^{68}Ga-labelled positron emission tomography (PET) probes for in-vivo study of tissue distribution and biological functions of αvβ8-integrin.
We developed a cyclic octapeptide with subnanomolar (IC_{50}=0.93 nM) affinity for αvβ8 and a high selectivity (IC_{50} for all other RGD-binding integrins >210 nM). On this basis, we synthesized monomeric and trimeric triazacyclononane-triphosphinate (TRAP) chelator conjugates by means of click-chemistry coupling. The Ga-68-labelled compounds were characterized in vitro and evaluated in-vivo, using SCID mice bearing subcutaneous MeWo (αvβ8+ human melanoma) xenografts, by μPET imaging and ex-vivo biodistribution 60 min p.i.. Expression density of αvβ8-integrin was assessed by β8-immunohistochemistry (IHC) for tumor and various other tissues.
The trimer exhibits a 37fold higher αvβ8-integrin affinity than the monomer (IC_{50}=0.89 vs. 33 nM, respectively) resulting in a higher tumor uptake (1.9±0.3 vs. 1.0±0.3 %ID/g, respectively, in biodistribution), while blockade resulted in the same baseline (0.25±0.16 and 0.25±0.09 %ID/g, respectively). Both tracers are excreted renally, consistent with high polarity (logD = –3.1±0.1 and –3.9±0.1, respectively). IHC showed intermediate β8-expression in the tumor while most organs and tissues were found β8-negative, resulting in low uptakes (<0.4 %ID/g) confirming a low degree of unspecific binding. A slight β8-expression in the lung was detectable with the trimer but not with the monomer (0.68±0.19 %ID/g vs. 0.35±0.16 %ID/g, respectively), showcasing a higher sensitivity of the

trimer. Favorable tumor/tissue ratios (t/blood: 6.7 and 5.5; t/liver: 6.8 and 6.6; t/muscle: 29 and 24 for trimer and monomer, respectively) are reflected by PET images with low background and excellent delination of the αvβ8-positive tumor.
In conclusion, both αvβ8-integrin-targeted probes possess favorable pharmacokinetics and are suitable for mapping αvβ8-integrin in vivo by means of PET, while the trimer is preferred due to higher sensitivity and higher absolute uptake in αvβ8-positive tissues.

3.2 Medicinal Chemistry and drug design

POS.15

Quantification of hydrolysed peptides and proteins by amino acid fluorescence

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Reliable quantification of peptides and proteins is essential for drug discovery. Screening current literature on drug discovery for information about quantification of tested peptides absolute values are often missing. In here, we describe the successful development and validation of an accurate and broadly applicable reversed phase high performance liquid chromatography coupled to fluorescence detector for the quantification of the aromatic amino acids phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr). Detection by fluorescence renders this method selective for determination of Tyr, Phe and Trp, with lowered detection limits. Using ion-pair chromatography, fluorescent amino acids were baseline separated under isocratic conditions on a C8 column within ten minutes. Peptides and proteins were hydrolysed before using 6 M HCl at 107 °C to yield the monomeric building blocks. Various protecting agents, like cysteine or ascorbic acid, were tested to ensure tryptophan stability. The developed method accurately (>95%) quantifies all fluorescent amino acids. The power of the method was confirmed by correct quantification of protein reference standard to 98.6% over all fluorescence traces. Applying this method to current research projects, pre-analytical differences between the nominal and actual concentrations of 12 peptide solutions were identified. Salt formation, residual solvents, weighing errors and other pre-analytical pitfalls resulted in noteworthy differences of up to 85% between the indicated and actual concentration of peptide solutions. Consequently, interpretation of activity data should be done carefully. Finally, only one solution is needed to perform UV-purity testing, quantification and activity testing.

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1. Allenspach, MA et al., *Journal of Peptide Science*, **2018**, xxx, xxx-xxx

POS.16

Hit-Discovery of Novel Bacterial Sliding Clamp DnaN Inhibitors by Virtual and Biophysical Screening Approaches

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The quest for novel classes of antibiotics acting with unprecedented mechanisms of action has become an obligation to combat the global threat of antibiotic resistance [1]. In this context, the β subunit of bacterial DNA polymerase III (the sliding clamp DnaN) can be regarded as a promising antibacterial target [2–4]. It is essential for DNA replication, structurally conserved in bacteria, yet different from the eukaryotic analogue permitting efficient, broad spectrum, and selective antibacterial activity. Moreover, derivatives of the natural antibiotic griselimycin targeting DnaN displayed a low rate of resistance development [2]. However, the discovered DnaN inhibitors so far suffer from weak or limited antibacterial effects [2–4]. Herein, we address the discovery of new chemical scaffolds targeting DnaN via virtual and biophysical screening strategies involving kinetic target-guided synthesis (KTGS). For the virtual screening (VS) approach, we established a workflow that was applied to a commercially available chemical database (1.3 M compounds). After exclusion of PAINS and Lipinski's rule of 5 violations, structure-based VS with a pharmacophore filter was performed using the co-crystal structure of Mycobacterium tuberculosis DnaN and cyclohexylgriselimycin [2]. Preliminary hits were clustered and cluster heads were selected for the evaluation of DnaN binding as well as antibacterial activity. Hits with promising antibacterial and cytotoxicity profiles were identified. Validated hits were prioritized according to their thermodynamic signatures using isothermal titration calorimetry (ITC) to select the most appropriate scaffold for optimization. In a second approach, we pursued a fragment-based screening through a modified three-step biophysical screening cascade [5]. Microscale thermophoresis (MST) technique was used for primary screening, and surface plasmon resonance (SPR) and ITC for hit validation. In the next step, co-crystallization of the best hits with DnaN will be carried out to characterize the binding mode and set the stage for fragment growing. Our new hits obtained by both hit-identification strategies establish the basis for the development of new antibiotic classes that could overcome the resistance problem.

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POS.17

Heterobivalent carboline derivatives as potential multi-target directed ligands for the therapy of neurodegenerative diseases

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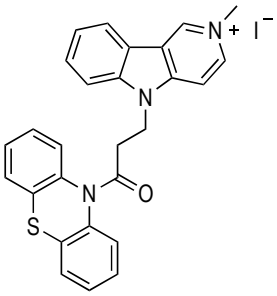
Neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease pose a major challenge to health systems in the near future. Therefore, the investigation of active compounds against established or new clinical targets for the treatment of neurodegenerative diseases is worthwhile. Due to the multifactorial nature of neurodegenerative diseases, it may be beneficial to address several targets with a single multi-target drug for efficient medication management. Therapeutic agents targeting either *N*-methyl-D-aspartate receptor (NMDAR), acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and monoaminoxidases (MAO) are well established in the clinic. Recently developed homobivalent carbolinium derivatives consisting of two linked gamma-carboline (5*H*-pyrido[4,3-*b*]indole) scaffolds were previously found to possess simultaneous inhibitory activity against all these targets [1].



Lead structure

Here, we present a structure-activity relationship study of novel heterobivalent compounds derived from a scaffold combining gamma-carboline with phenothiazine as lead structure. The compounds were synthesized and tested for their inhibitory activities on AChE, BChE, MAO-A, and MAO-B. Furthermore, the potency to inhibit NMDA receptors was investigated in a cell-based functional NMDA receptor assay. Further studies will focus on the derivatization of compounds that showed a promising pharmacological profile in bioassays and investigation of their metabolic stability and toxicity.

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POS.18

Nitroreductase-Mediated Prodrug Activation to Target Epigenetic Enzymes in Cancer Cells

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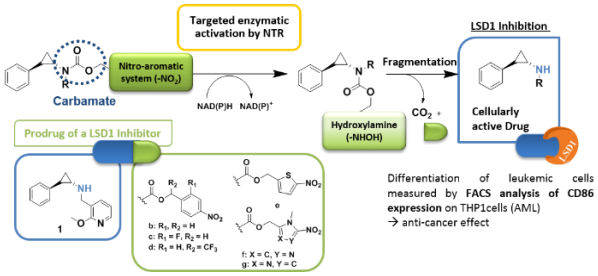
So far, known small molecule inhibitors of epigenetic proteins lack selectivity in affecting only tumour cells, often resulting in acute damage to healthy and rapidly cycling cells. The specific targeting of tumor cells should increase therapeutic effectiveness and decrease toxic side effects during treatment. As showcase model for the use of bacterial Nitroreductase (NTR) in enzyme-prodrug systems for epigenetic targets, the lysine-specific demethylase 1 (LSD1 or KDM1A) was selected.

To achieve target-specificity, pharmacologically inactive and nontoxic forms of known LSD1 inhibitors with a nitro-aromatic system, so-called *bioreductive prodrugs*, are designed, synthesized and tested against LSD1 activity *in-vitro* and on cultured AML THP1 cells. The LSD1 inhibitors are protected by a carbamate linked to the nitro-aryl bioreductive system which is reduced by the NTR, leading to subsequent release of the active drug.

We identified promising prodrug/drug pairs by measuring the expression of CD86 surface marker and by performing colony-forming unit assays with THP1 cells.[1] Several prodrugs are converted into the active parent drug by the NTR, which is solely expressed in transduced tumour cells. Depending on the nitro-aryl system, different activation patterns can be observed both *in vitro* and *in vivo*. By applying different targeting techniques such as antibody-directed enzyme-prodrug therapy (ADEPT) and gene-directed enzyme-prodrug therapy (GDEPT),[2] these prodrugs provide a direction for more selective anti-cancer drugs.



We thank the Deutsche Forschungsgesellschaft for funding by CRC992 Medical Epigenetics, Project A04

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POS.19

[*b*]-Annulated halogen-substituted indoles as potential DYRK1A inhibitors

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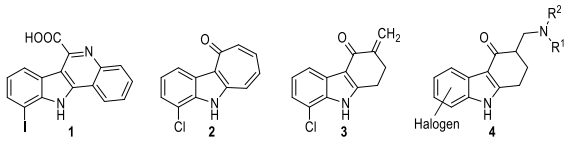
The gene for DYRK1A is located on chromosome 21 in the Down syndrome critical region, and thus 1.5 fold overexpressed in this disease. Besides its regulatory function regarding the cell proliferation and differentiation, DYRK1A plays a key role in elementary cell processes like splicing, apoptosis and neurogenesis.[1] Therefore, DYRK1A is a promising target in the treatment of Down Syndrome.[2] 10-Iodo-11*H*-indolo[3,2-*c*]quinoline-6-carboxylic acid (**1**) is a potent and selective inhibitor of DYRK1A[3], but suffers from poor physicochemical properties and has a rather high molecular mass. In order to improve these properties, smaller analogues **2** and **3** of compound **1** were designed. While **2**[4] and **3** showed less potency compared to **1**, their solubility was not significantly improved. Based on docking studies applying an X-ray structure of DYRK1A in complex with inhibitor **2**, derivatives of **4** containing basic side chains were designed and synthesized. Indeed, **4** showed improved aqueous solubility but even lower potency than **2** and **3**. The results of synthesis, docking studies, biological evaluation and solubility studies will be presented.



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POS.20

Ferroptosis as new approach in cancer treatment – impact of iron salophene complexes

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Abstract Ferroptosis appears as a promising strategy for cancer therapy because it is associated with tumorigenesis. Moreover, activation of regulated cell death reveals a fundamental and beneficial approach for cancer treatment. [1]

Ferroptosis is an iron-dependent form of non-apoptotic cell death, which results in the accumulation of reactive oxygen species (ROS). Catalysis of the lipid peroxidation and the consequent arising of damaging radicals is feasible by redox-active iron ions according to the Fenton's reaction. [2] Thus, sufficient availability of cellular iron is essential, whereas uptake is obtained by binding of ferrous compounds to transferrin and subsequent endocytosis via the transferrin receptor 1. [3]

Iron complexes bearing Schiff-base ligands such as the representative [*N,N'*-bis(salicylidene)-1,2-phenylenediamine]chloridoiron(III) [Fe(III)salophenCl], are considered to cause their effects by induction of ferroptosis. In order to optimize its pharmacological profile, various derivatives of [Fe(III)salopheneCl] were synthesized and tested for anti-leukemic effects using different leukemic cell lines.

The compounds concentration-dependently reduced the proliferation and the metabolic activity of all examined cell lines. Furthermore, they induced late apoptosis / necrosis and the generation of ROS. The cellular effects of the complexes were completely abolished upon simultaneous administration of the necrosis or ferroptosis inhibitors Necrostatin-1 or Ferrostatin-1, respectively.
The involvement of ferroptosis in the mode of action is unique since it is yet described as effect of $Fe^{2+/3+}$ ions. To exclude iron release from the complexes, stability was studied under physiological conditions on the example of [Fe(III)salophenCl], whereby no degradation even in the presence of Ferrostatin-1 or the iron chelator deferoxamine took place. Moreover, the extent of [Fe(III)salophenCl] to bind apo-transferrin was quantified and revealed a high degree of binding, thus indicating a transferrin-mediated uptake.
In conclusion, induction of ferroptosis can be suggested as a new approach for anti-leukemic treatment as demonstrated with the present iron salophene complexes.

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POS.21

Mutated variants of 17beta-Hydroxysteroid dehydrogenase type 14 Studying the functional role of residues next to the catalytic center of the enzyme

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17beta-Hydroxysteroid dehydrogenase type 14 (17β-HSD14) catalyzes the oxidation of the highly potent 17β-Hydroxysteroids like Estradiol, 5-diol, or Testosterone. Thereby, the enzyme regulate the local concentration of the active hormones in the tissue. The enzyme was found to be dominantly present in the brain, liver, and placenta with two natural variants (S205 and T205). It might, therefore, play an important role in metabolic pathways in these tissues. First potent inhibitors have recently been identified for the human enzyme by our working group, also the crystal structures of the corresponding ternary enzyme-cofactor-inhibitor complexes have lately been identified using X-ray crystallography.

1) The biochemical characterization of the wild types and the mutated variants were performed by a fluorimetric assay to better understand the functional role of individual residues, which in some cases are conserved or replaced across the narrow family of 17beta-Hydroxysteroid dehydrogenase, we embark onto a mutational study. The different mutated variants have been characterized by enzyme kinetics and crystal structure analysis. The mutant crystals are crystallized in complex with nonsteroidal inhibitor using the co-crystallization method. This new structural information will assess the involvement of the mutated amino acids in the catalytic process and in the stabilization of the inhibitor and will highlight the role of the dimer formation for enzymatic activity.

2) Additionally, the experiments will also shed some light on the importance of oligomerization of the protein for enzymatic activity.

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POS.22

Boosting Anti-inflammatory Potency of Zafirlukast by Designed Polypharmacology

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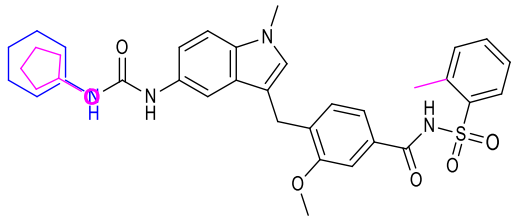
The multifactorial nature of complex diseases such as chronic inflammation often demands multiple pharmacological interventions for a stable treatment with sufficient therapeutic efficacy [1]. However, excessive combination of multiple drugs has several drawbacks. Multitarget design is a powerful strategy to avoid such polypharmacy and to retrieve bioactive small molecules with high therapeutic efficacy and safety. Simultaneous modulation of rationally selected synergistic biological targets may allow moderate modulation of the individual targets to retain robust therapeutic effects. To generate an anti-inflammatory multi-target agent, we selected the cysteinyl leukotriene receptor 1 ($CysLT_1R$) [2,3] antagonist zafirlukast (**1**) [4] as lead compound. We have discovered moderate off-target activity of Zafirlukast on the peroxisome proliferator-activated receptor γ (PPARγ) [5] and the soluble epoxide hydrolase (sEH) which like $CysLT_1R$ are involved in inflammatory processes and like $CysLT_1R$ interact with metabolites of arachidonic acid. All three molecular targets hold great anti-inflammatory potential and their selective modulation reduced acute inflammation in animal models [6,7].

The multi-target optimization strategy of zafirlukast was supported by *in silico* binding mode analysis which suggested that minor structural modifications were sufficient to enhance PPARγ agonistic and sEH inhibitory activity. Guided by these observations, we have developed a close analogue (**2**) of zafirlukast (**1**) by three minimal structural changes that comprises remarkably improved potency on PPARγ (EC_{50}= 0.30 µM ±0.02; 36±1 max. rel. activation) and sEH (IC_{50}= 0.043 µM ±0.002). In an animal model of acute inflammation (paw edema model in mice), the triple modulator **2** exhibited robust anti-inflammatory activity and was significantly superior to the lead compound (**1**).

In conclusion, we have successfully employed an approved drug to design a triple modulator with improved anti-inflammatory activity while preserving the lead compounds structural properties, molecular weight and favorable pharmacokinetic profile. This combined strategy of multitarget design and selective optimization of side-activities [8] may hold enormous potential for future drug discovery, especially in times of increasing polypharmacy.



Zafirlukast (**1**) / triple modulator **2**

pEC_{50}(PPARg) = **5.6** / **6.5**, pIC_{50}(sEH) = **5.7** / **7.4**, pA_2($CysLT_1R$) = **11.4** / **8.7**

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POS.23

Design and synthesis of selective HDAC6 inhibitors as potential anti-tumour agents

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In recent years, histone deacetylase (HDAC) inhibitors have been well-established as a new class of epigenetic anti-tumour agents. Several drugs, e. g. vorinostat, panobinostat, romidepsin, and belinostat have reached FDA-approval for the treatment of non-solid cancers while numerous further compounds are currently in clinical trials for the treatment of several cancer types and other diseases such as neurodegenerative disorders and HIV.[1]

HDACs are enzymes divided into classes I-IV of which eleven zinc-dependent isoforms are known.[1] Depending on the respective isoform and its substrates, several anticancer effects of HDAC inhibitors have been identified. *Pan*-inhibitors which inhibit multiple isoforms show high antitumour activity but also severe adverse effects which limit their scope to oncological applications.[1] The class IIb isoform HDAC6, however, induces the aggresome function and modulates the activity of α-tubulin and Hsp90, thus allowing antitumour activity through accumulation of misfolded proteins accompanied by minor side effects.[2] Consequently, HDAC6 has been identified as a promising target for isoform-selective inhibitors.[1,2]

In 2016, the first crystal structure of human HDAC6 revealed distinct features [3] which facilitate the design of selective inhibitors despite the highly-conserved structures. First attempts to design HDAC6-selective drugs have since been made and two inhibitors, ricolinostat and its derivative citarinostat, are currently being evaluated in clinical trials against solid and non-solid tumors.

Following previous works on α-peptoid scaffolds as selective HDAC6 inhibitors, we herein report the design, diversification and synthesis of tetrazole-substituted inhibitors.[4] The library synthesis was performed via the highly versatile Ugi-azide 4-component reaction which yielded the core scaffolds of all final compounds within one step (40-84%). Further diversification of some derivatives was achieved by acylation using commercial or *in-situ*-made acyl chlorides (53-87%) before final hydroxylaminolysis of all precursors gave the desired hydroxamic acids **9a-l** in 28-95% yields (Figure 1). All synthesised final compounds were evaluated in regards to their HDAC6 selectivity profile. Their inhibitory activity against HDAC1 and HDAC6 was determined in a biochemical assay using recombinant HDAC isoforms and ZMAL (Z-(Ac)Lys-AMC) as substrate. Strikingly, most compounds showed potent inhibitory activity against HDAC6 and significant selectivity over HDAC1.

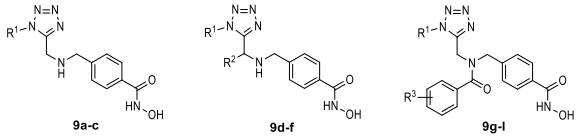

Figure 1. Structures of tetrazole-capped HDAC6-selective inhibitors **9a-o**.

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POS.24

Synthesis and evaluation of peptide-based chelators for the development of novel ^{89}Zr-PET radiotracers

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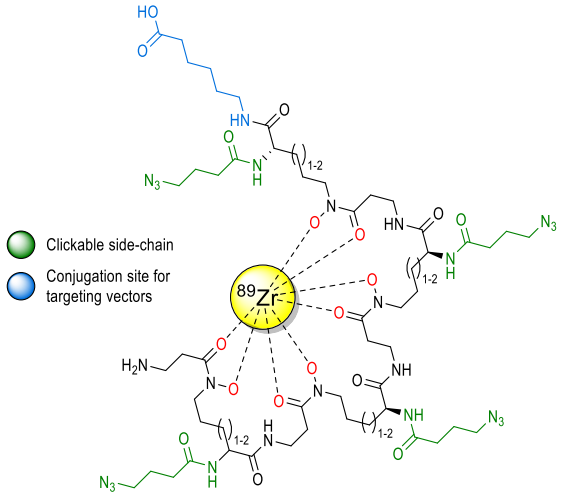
Over the past decade the *β*-emitter ^{89}Zr has emerged as a promising radioisotope for positron-emission tomography (PET). Due to its facile production and beneficial decay properties ($t_{1/2}$ = 78.4 h, $E_{β\text{-max}}$ = 901 keV), it is a perfect match for antibody- and small protein-coupled radiopharmaceuticals that have biological half-life times of several days [1]. Despite these advantages the use of zirconium is accompanied by several challenges: Zirconium forms highly charged ions (Zr^{4+}) with a small ion radius (59-89 pm) making hard donor chelators with coordination numbers of 4-9 a necessity [2,3]. One of the most extensively studied chelators for zirconium is desferrioxamine B (DFO), a hydroxamate based siderophore found in the bacterium *Streptomyces pilosus* [4]. However, several studies showed a limited complex stability of ^{89}Zr-DFO conjugates *in vivo*, resulting in reduced S/N-ratios and accumulation of the radiometal in bone tissue [5]. Because of these shortcomings, tremendous effort has been put into the development of new zirconium chelators [6]. Recent studies have proven that an octadentate coordination scheme found in novel DFO-derivatives such as DFO* and DFO-squaramide esters is superior to the hexadentate nature of DFO [7,8].
We herein report the synthesis of a series of modular peptidic DFO-analogues bearing four hydroxamate functionalities and its zirconium complexes. The ligand is synthesized via facile peptide coupling from a monomeric building block derived from L-lysine or L-ornithine coupled to *β*-alanine to mimic the natural compound. The modular approach allows the introduction of additional side-chains bearing azides, and thus enables orthogonal modification of the ligand *via* copper-catalysed "*click-chemistry*".



Acknowlegments: We want to gratefully acknowledge financial support from the NIH (PCQ5 grant).

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POS.25

Tuning the nuclear receptor selectivity of lipid-lowering agent Wy14,643

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Wy14,643 was first published in the mid-1970s as a new agent with good lipid-lowering effects in rodent models, but without knowledge of its molecular target [1]. Later, it was identified as a dual agonist for the peroxisome proliferator activated receptor (PPAR) alpha and gamma [2]. Since its discovery it was extensively used as a tool compound to investigate PPAR mediated effects resulting in the discovery of a variety of beneficial effects in various diseases [3]. But although these promising findings and the fact that its lipid lowering efficacy is similar to that of fibrates, Wy14,643 has never reached clinical trials, which might be explained by multiple side-effects [4]. We discovered that Wy14,643 also robustly activates the retinoid X receptors (RXR) alpha, beta and gamma, which gives a sound explanation of its pleiotropic effects. RXRs possess a unique role amongst nuclear receptors as universal heterodimer partners for other ligand-activated transcription factors and permissive RXR heterodimers are involved in multiple physiological functions. Moreover, recent in vivo observations characterize RXRs as potential molecular target to treat neurodegenerative diseases such as Alzheimer's [5] and Multiple Sclerosis [6]. To further explore this promising therapeutic potential, novel drug-like RXR modulators are needed.

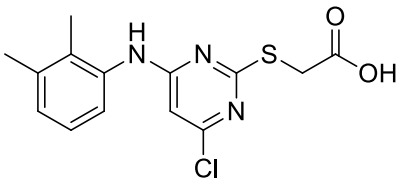

Fig. 1: Structure of Wy14,643

To decipher the structural determinants for Wy14,643 mediated activation of PPARs and RXRs, we have systematically varied the scaffold and its substituents and characterized the derivatives in specific cell-based hybrid reporter gene assays for all PPAR and RXR subtypes. We observed specific differences in the structure-activity relationship (SAR) on both receptor types and identified key structural elements driving selective or dual potency. Especially variation of the chlorine substituent of the central pyrimidine ring and the 2,3-xylen moiety had a marked impact on potency. Furthermore, we improved the reactivity of the lead structure by replacing the sulphur and identifying suitable substitutes for the chlorine.

Our findings on one hand give a sound explanation for the pleiotropic effects of Wy14,643 and on the other hand implicate an interesting SAR profile with specific differences for both targets. This knowledge could be used to decrease the amount of adverse side-effects, mediated by RXR but also render Wy14,643 as a valuable lead compound for selective RXR modulator development.

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POS.26

Enhancing metabolic stability of dual farnesoid X receptor and soluble epoxide hydrolase modulators for the treatment of non-alcoholic steatohepatitis

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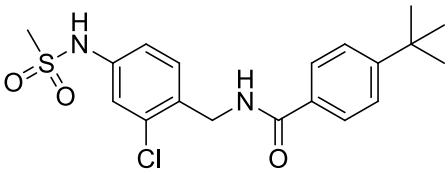
Global epidemics of obesity originating from western diet and lifestyle are accompanied by a burden of non-alcoholic fatty liver disease (NAFLD) and a subsequent non-alcoholic steatohepatitis (NASH). Long-term consequences of NASH are severe because it can progress to cirrhosis, hepatocellular carcinoma and end-stage liver disease. The prevalence of NAFLD is increasing both in Europe and the United States of America which makes NASH and NAFLD one of the leading causes of chronic liver disease and liver transplantation [1,2,3]. Nonetheless there is still no satisfying pharmacological option to counteract the progression of NASH. Despite no drug has been approved, yet, a variety of biological targets has been validated for NAFLD/NASH treatment in animal models and clinical trials. In the light of the multifactorial nature of NASH, we have developed a dual modulator of FXR and sEH to simultaneously exploit the clinical efficacy of FXR activation to reduce NASH progression and the hepatic anti-inflammatory effects of sEH inhibition [4,5].



dual FXR/sEH modulator
EC_{50}(FXR) = 0.0204 ± 0.0042 µM
IC_{50}(sEH) = 0.0041 ± 0.0004 µM

The dual modulator revealed promising therapeutic efficacy in toxin and diet induced NASH rodent models proving the dual strategy successful. However, its *in vivo* profile displayed high oral bioavailability and rapid uptake but only a modest half-life [4,5]. To address this pharmacokinetic issue, we analyzed the metabolic conversion of the dual modulator and characterized its main metabolites. We discovered that the compound is mostly degraded by sulfonamide hydrolysis and hydroxylation of the *tert*-butyl residue. Using that information, we synthesized analogues of the dual compound aiming to prevent hydrolysis and hydroxylation with suitable structural modifications while maintaining high dual potency. We succeeded in interfering with the metabolic degradation and designing dual modulators with improved metabolic stability and favourable activity as dual modulators of FXR and sEH.

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POS.27

Improvement of a dual FXR/PPARα agonist for the treatment of NASH

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Non-alcoholic fatty liver disease (NAFLD) is characterised as hepatic lipid accumulation (steatosis) in the absence of pathologies such as viral hepatitis or alcohol abuse [1]. The disease progresses to non-alcoholic steatohepatitis (NASH) when hepatic inflammation, hepatocellular injury and hepatocyte ballooning occur [2]. Finally, fibrosis, cirrhosis, or hepatocellular carcinoma (HCC) possibly arise [3,4].

Since NAFLD is considered as hepatic manifestation of the metabolic syndrome and peroxisome proliferator-activated receptors (PPARs) are involved in many diseases associated with the metabolic syndrome, NAFLD is another potential field of application for PPAR modulators. However, so far only the PPARα/δ agonist elafibranor has revealed efficacy in the GOLDEN-505 trial [5], while single modulation of the PPAR subtypes PPARα and PPARγ have disappointed [6]. Another promising candidate for the treatment of NAFLD and NASH is obeticholic acid. Obeticholic acid is a synthetic bile acid, which stimulates the bile acid receptor FXR. During the FLINT trial reduced hepatic steatosis and fibrosis could be determined [7].

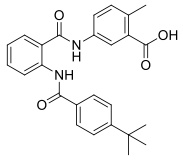

Fig. 1: lead structure

Because dual agonism seems to be beneficial and the results from the clinical development of obeticholic acid and elafibranor suggest that FXR and PPARα activation have crucial importance in NASH treatment, we decided to investigate the dual activation of PPARα and FXR. PPARα transcriptionally regulates multiple metabolic processes including β-oxidation, lipid transport and gluconeogenesis and is also involved in inflammatory processes [8]. Activation of FXR decreases gluconeogenesis and the amount of circulating triglycerides accompanied by improved insulin sensitivity [7]. Altogether the dual activation of both receptors could have synergistic effects in NASH treatment. The lead structure that was selected already comprises low EC_{50} values on both targets (EC_{50}(PPARα) = 0.21 µM, EC_{50}(FXR) = 0.045 µM) but its solubility is very limited (aq. solubility = 2.1 mg/L) and has to be improved [9]. For this purpose, we replaced the highly lipophilic tert-butyl group with more hydrophilic residues, mainly aliphatic amine or cyclic amine components. Substitution with piperidine produced a potent dual FXR/PPARα agonist with significantly improved solubility (EC_{50}(PPARα) = 0.09 µM, EC_{50}(FXR) = 0.24 µM, aq. solubility = 5.0 mg/L). In contrast, larger cyclic amines seemed not to fit into the binding pocket of PPARα. Further SAR studies are implementation of hetero atoms in the aromatic rings of the scaffold, replacement of the methyl group by more polar residues and we address the carboxylic acid side chain at the head group for further improvement of dual activation of FXR and PPARα as well as solubility.

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POS.28

Ruthenium(II) *N*-heterocyclic carbene complexes: Biological evaluation as potential antitumor and antibacterial agents

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Ruthenium complexes have been in the focus of anticancer metallodrug research over the last years and clinical trials on interesting drug candidates are ongoing. Nevertheless, the development of new structures and investigations on structure-activity-relationships are of high interest. [1,2]

We have been studying N-heterocyclic carbenes (NHCs) as stable ligands for new metal based drugs and explored their medicinal chemistry and structure-activity-relationships.[3] For example, regarding complexes with ruthenium(II) large aromatic side chains seem to be important to induce the necessary lipophilicity for entering the cells and to induce potent cytotoxic effects. Mechanistic studies indicated that the mode of action of Ru(II)-NHC complexes is based on interactions with selenol- and cysteinol-containing biomolecules (e.g. thioredoxin reductase, TrxR). [4,5]

In order to shed more light on the medicinal chemistry and chemical biology of Ru(II)-NHC-complexes, especially regarding ligand- and metal-based effects, a series of new complexes has been prepared and the biological evaluations have been extended broadly including antibacterial screening assays. [Figure 1] The complexes as well as the metal free imidazolium precursors showed cytotoxic effects on cancer cells in the low micromolar range, however, only the ruthenium

complexes triggered good activity against gram-positive bacterial strains. Recent results on cytotoxicity, cellular uptake and TrxR inhibition of ruthenium(II)-NHC-complexes will be presented on the poster and new insights into structure-activity-relationships will be discussed.

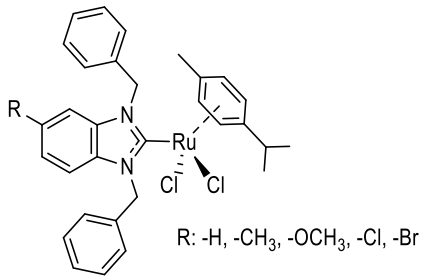

Figure 1: Newly synthesized ruthenium(II) NHC complexes

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POS.29

Synthesis and evaluation of phosphonic acids for the antimicrobial coating of metal surfaces

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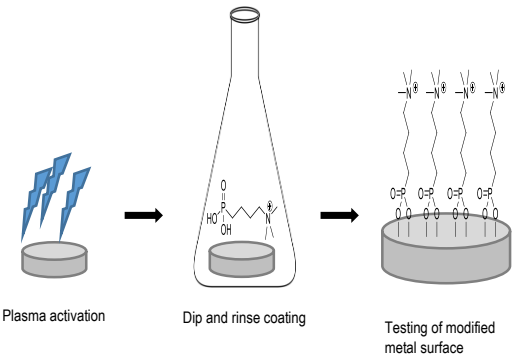
The increasing emergence of multiple drug resistance (MDR) in bacteria and consequently the declining effectivity of antibiotics demand the development of new antimicrobial strategies [1]. As a matrix for the proliferation of bacteria, surfaces in hospitals, sanitary installations or other public places pose a huge risk [2]. Bacterial growth often occurs via the formation of a protective biofilm within the bacterial colony, which renders treatment other than mechanical removal futile [3]. Next to the synthesis of novel antibiotics or disinfectants, the coating of metal surfaces with molecules that prevent the formation of biofilms is a promising addition to the antimicrobial arsenal. This approach looks particularly appealing when it comes to the biofilm formation on invasive tools, such as surgery equipment, catheters or implants [4].

Passive and active antifouling approaches can be differentiated depending on the point of interaction within the biofouling process. While passive approaches suppress the reversible binding of biological matter to the surface, thereby impeding bacterial growth due to a lack of nutrients, active approaches aim at directly killing microbes via the release of toxins or cell lysis [5].

Quaternary alkyl ammonium salts have proven to be effective contact biocides by destroying the bacterial membrane through various ionic interactions [6]. As anchoring group, phosphonic acids exhibit many favourable properties such as robust synthetic approaches, strong binding to a high number of metals, pH independency as well as high stability [7].

Herein we present the synthesis of cationic as well as zwitterionic phosphonic acids bearing different organic residues. The modular synthetic approach is meant to allow easy modification as well as access to a library of different substances in a low number of steps from cheap and easily available starting materials. The synthesized molecules are then coated on different medicinally relevant materials such as titanium, aluminium or stainless steel. Afterwards, we present the results of the biological testing of the antimicrobial activity against different strains of bacteria.



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POS.30

Comparative Analysis of Fragment Binding Using Orthogonal Biophysical Methods Yield Surprising Results

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X-ray crystallography provides structural information that is crucial for fragment optimization, however there are several criteria that must be met for a successful fragment screening including the production of soakable and well-diffracting crystals. Therefore, having a reliable cascade of screening methods to be used as pre-screens before X-ray crystallography is beneficial. There are several biophysical methods developed to rapidly identify weakly binding fragments to a target protein. But the question remains; how many of the hits from these methods are false negatives? Are these screens missing fragments that are potential hits? In a follow-up study to investigate whether different screening methods will reveal similar collections of putative binders, 71 endothiapepsin X-ray fragment hits[1] were rescreened for a second time with STD-NMR, where the difference between the initial and second STD-NMR was a lower concentration of buffer, and the absence of salts. The fragments were screened in the absence and presence of Ritonavir; a potent active site inhibitor used to confirm binding to the active site via competitive displacement of the fragment. The resulting overlap with the previous screen was 69% showing that even slight changes in buffer concentration can lead to a different hit outcome. Interestingly, the results of the STD-NMR also revealed binding of active site fragments, even in the presence of Ritonavir, suggesting that both ligands bind simultaneously. X-ray crystallography was used to map the structural details of this interaction. For this endothiapepsin crystals were soaked with solutions of fragment alone, as well as fragment and Ritonavir. The

results revealed some structures showing a relocation of active site fragments to new binding sites in the presence of Ritonavir blocking the active site. The relocation of the fragment binding site could not have been predicted based on the results of the STD-NMR alone. As every biophysical assay has its specific limitations such as buffer selection pH and acceptable range of fragment concentration, trying to maintain similar conditions across the assays is difficult. This made us question whether we could also use X-ray crystallography for the pre-screening of fragments. We propose the use of a fluorescent reporter ligand that binds in the active site, in turn imparting fluorescing properties to the crystal visible under UV light (366nm). Upon soaking of the crystals into active site fragments, we can predict instantly whether a fragment has displaced the reporter ligand in the active site based on the change in fluorescence of the crystal.

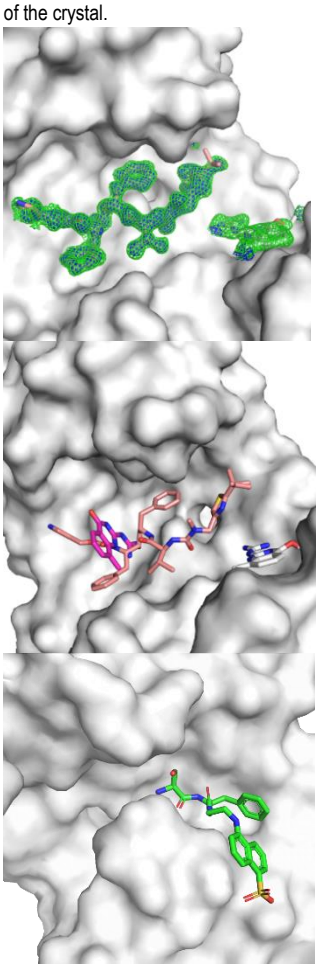

Figure: Endothiapepsin crystals soaked in Ritonavir and an active site fragment yield difference electron density maps for both compounds (first). In the new structure (center), the fragment shows a new binding mode (white) relative to its original binding mode in the active site (purple). The fluorescent reporter ligand is on the last figure..

We acknowledge the beamline support of Bessy in Berlin and ESRF in Grenoble, for practical help. We also thank the HZB for travel grants and the EU for ITN AEGIS funding. We also thank Radeva N., Schiebel J., Koester H., and Metz A. for their original work on the project.

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POS.31

Identification of intracellular binding partners of platinum drugs in ovarian and colorectal cancer cells by combining two-dimensional gel electrophoresis and BODIPY-tagged drug analogues

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The major drawback of platinum-based chemotherapy is the development of resistance in the course of treatment. It is known, that only small amounts of intracellular platinum reach the nucleus and can thus interact with DNA leading to apoptosis. However, the fate of the remaining drug and the relevance of the alternative binding partners for tumor cell sensitivity and resistance remain poorly elucidated.

The aim of this project is to compare binding partners of cisplatin and oxaliplatin in sensitive and drug-resistant cells using fluorescent derivatives of these platinum drugs featuring the BODIPY dye (BDP-cisplatin and BDP-oxaliplatin).

For this purpose, we use sensitive and cisplatin-resistant ovarian cancer cell lines A2780/A2780cis and sensitive and oxaliplatin-resistant colorectal cancer cell lines HCT-8wt/HCT-8ox. These two entities are of particular interest, since cisplatin is used in ovarian cancer while oxaliplatin is a part of the first line treatment of colorectal cancer. By MTT-based cytotoxicity tests we have determined the anticancer activity of fluorescent analogs and the respective parent compounds. After treatment of cells with the BODIPY-tagged platinum complexes and subsequent gel electrophoresis, the targets of platinum drugs have been investigated via mass spectrometry.

As expected, both colorectal cancer cell lines showed intrinsic resistance to cisplatin. In HCT-8 cells, the mean EC_{50} of the drug was 13,7 µM, in HCT-8ox it was 16,7 µM (in A2780 ovarian cancer cells EC_{50} = 0,96 µM and in A2780cis 13,7 µM). BDP-cisplatin was less cytotoxic than the parent drug (EC_{50} A2780 18,9 µM; A2780cis 98,8 µM; HCT-8: 99,2 µM; HCT-8ox 163,8 µM). Nevertheless, cisplatin-resistant cells were cross-resistant to BDP-cisplatin, which was not the case in oxaliplatin-resistant cells corresponding to the activity of cisplatin itself. BDP-oxaliplatin is currently being evaluated but given the earlier reports on fluorescent derivatives of platinum drugs similar results are expected [1,2]. Preliminary experiments suggest PDIA1 (protein disulfide-isomerase 1) as a binding partner of BDP-cisplatin, which is in agreement with earlier results [3].

BDP-cisplatin has been shown to mimic the biological behavior of the parent drug. After evaluation of BDP-oxaliplatin and successful identification of binding partners in both sensitive /resistant cell line pairs, we plan to characterize detected proteins and their impact on platinum drug sensitivity. Furthermore the comparison of this method to other detection approaches, such as immunoprecipitation and selective pull-down of target proteins after copper-catalyzed click reaction is ongoing.

Acknowledgements: We thank Prof. Ulrich Jaehde for fruitful discussions.

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POS.32

PROTAC-Induced Degradation of Sirtuin2 (Sirt2)

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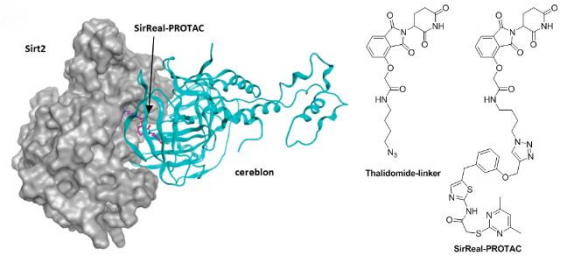
Proteolysis targeting chimeras (PROTACs) promote ubiquitination und proteasomal degradation of a target protein by recruiting E3 ubiquitin ligases. Previously it was shown that PROTACs with a thalidomide moiety are able to lead to degradation of selective targets. Thalidomide as a cereblon ligand effects the proximity to the ubiquitin ligase, while a second moiety of the PROTAC enables a selective binding of the target protein.[1, 2]
We have developed a PROTAC based on an azide-thalidomide-linker and the sirtuin rearranging ligands (SirReals), which are highly isotype-selective inhibitors for Sirt2, by forming a triazole-linked compound. We described for the first time a PROTAC formed by Cu(I)-catalyzed cycloaddition of an azido-thalidomide derivative to an alkynylated small molecule. This technique will allow further triazole-linked PROTACs for selected targets.



In HeLa cells, our SirReal-based PROTAC induced isotype-selective Sirt2 degradation that results in the hyperacetylation of the microtubule network coupled with enhanced process elongation. Thus, our SirReal-based PROTAC is the first example of a probe that is able to chemically induce the degradation of an epigenetic eraser protein.[3, 4]

Funding by Deutsche Forschungsgesellschaft (DFG) GRK1976 + Ju 295/13-1 + Ju 295/14-1.

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POS.33

Folic acid derivatives for prostate cancer imaging

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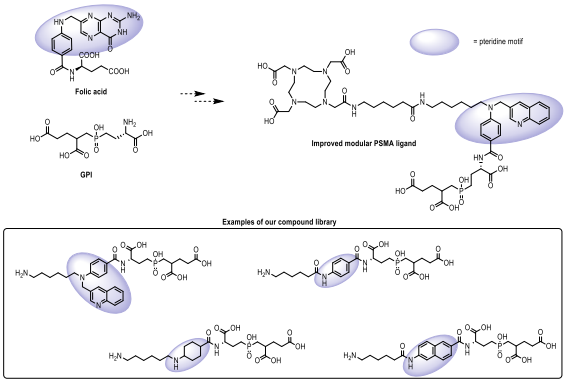
Folates are essential cofactors in the *de novo* biosynthesis of pyridine and pyrimidines [1]. Moreover, antifolates are key components in cancer therapy [2]. Targeting tumor specific cell surface epitopes, so called tumor markers, with small molecules can lead to improved tools for cancer diagnosis and therapy. Elevated levels of prostate specific membrane antigen (PSMA) are used as a tumor marker for prostate cancer [3]. PSMA is a glycosylated type-II membrane protein that is present in high density on the surface of malignant prostate cancer cells. Its expression increases with clinical stage, thus making it an extremely useful tumor marker [4]. Phosphinic acids like GP can be used as modular ligands for the targeting of prostate cancer [5]. GPI binds with nanomolar affinity to PSMA and permits conjugation of effector molecules like dyes without altering the binding properties [6]. However, GPI has suboptimal binding properties *in vivo* and needs to be improved for imaging applications in animals or humans. GPI has been developed as a transition state analogue of the native PSMA substrate *N*-acetylaspartylglutamate (NAAG). In addition, PSMA has been found to act as a folate hydrolase and does thus recognize folylpolyglutamates in the same binding pocket as NAAG [7]. We have combined properties of the known ligand GPI with structural elements of folates and a conjugation site for effector molecules (in our case DOTA). The poster highlights the design and synthesis of improved modular PSMA ligands. With this compound library we would like to evaluate the impact of different aromatic moieties as pteridine analogues, their spacing from GPI and the role of geometry and aromaticity of the spacer via *in vitro* assays using LNCaP (PSMA positive) and PC3 (PSMA negative) cell lines [8].



Acknowledgments: We want to gratefully acknowledge financial support from the NIH (PCQ5 grant).

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POS.34

γ-Ketoester as Key Intermediates for the Synthesis of γ-Hydroxybutenolides

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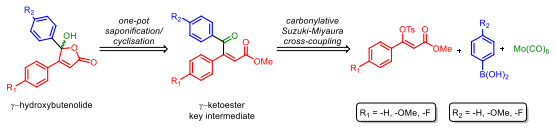
γ-Hydroxybutenolides are structural motifs, which occur frequently in natural products. This class of natural products exhibit a broad range of biological activities such as anti-inflammatory, antitumor, antimicrobial and inhibition of phospholipase A2 .[1] Furthermore, fully synthesized derivatives such as PD156707 by Doherty et al. gained interest as effective endothelin receptor type A (ETA) antagonists [2]. Moreover, these structures were applied as key building blocks for several natural product syntheses [3].

Due to the synthetic and medicinal relevance of these compounds, various synthetic approaches of highly functionalized structures were established [2][4]. Recently, we established a short and robust synthesis with high tolerance to functional groups. The key steps of the synthesis

are a $Mo(CO)_6$ mediated carbonylative Suzuki-Miyaura cross-coupling and a one-pot saponification-cyclisation.



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POS.35

Synthesis of new rubrolide derivatives

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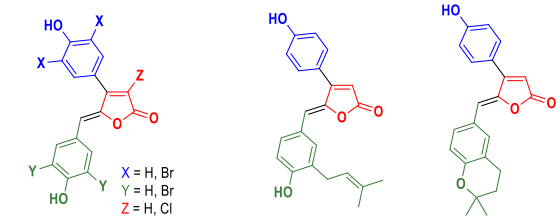
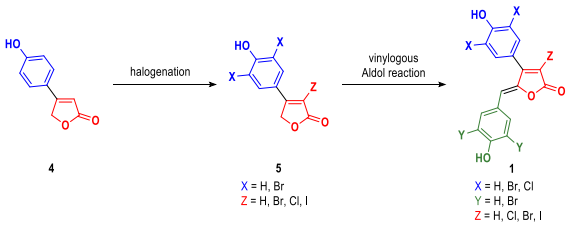
In 1991, the first rubrolides have been isolated from the marine fungus *Ritterella rubra*. This natural product class family has shown various pharmaceutical relevant activities such as moderate inhibition of protein phosphatases 1 and 2A, antibacterial, and antiviral effects[1] In 2014, rubrolide R **1** and S **2** have been isolated from *Aspergillus terreus*[2] and in 2017 the total syntheses of both compounds have been published.[3] These two natural rubrolides revealed biological activity against pH1N1 and H3N5 and rubrolide S **2** has also shown activity against the tobacco mosaic virus.[4]



General Rubrolide scaffold **1** | Rubrolide R **2** | Rubrolide S **3**

In order to increase the biological activity we investigated different halogenation patterns of the rubrolide scaffold. Herein, we present a general method for selective halogenation of privileged building block **3** by using various Lewis acids. With these halogenated building blocks **4** novel rubrolide derivatives can be synthesized by subsequent vinylogous aldol condensation.



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POS.36

Total Synthesis of Scleropentaside A

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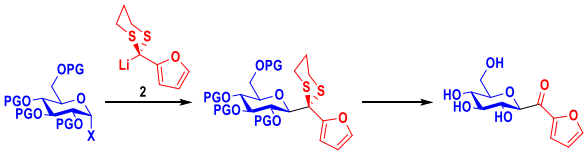
In 2012, Scleropentasides have been isolated from the leaves and twigs of *Scleropyrum pentandrum*. Scleropentasides are b-*C*-glycosidic furan-2-carbonyl compounds of D-glucose. These kind of *C*-glycosidic compounds have not been isolated from natural sources before and are unique in terms of their structural pattern so far. In terms of biological activities only the antioxidative properties of Scleropentasides have been investigated so far.[1] A synthetic approach to the Scleropentaside natural product family would help to obtain more material for further biological evaluation which is necessary to evaluate this interesting natural product class and derivatives thereof in detail.



For the synthesis of Scleropentaside A **1** a β-selective type of *C*-glycosylation was achieved. The reaction of lithiated dithianes **2**, which were firstly introduced by Corey and Seebach as nucleophilic carbonyl synthons[2]; with glycosyl halides **3** as electrophiles yielded *C*-glycosidic compounds **4** in good yields with excellent β-selectivity. Scleropentaside A **1** was synthesized by this approach in just three linear steps starting from D-glucose.

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POS.37

Novel diflapolin derivatives as sEH/FLAP inhibitors

Schoenthaler, M.[1]; Vieider, L.[1]; Edlmair, S.[1]; Schaffenrath, T.[1]; Widmann, I.[1]; Zöller, E.[1]; Kretzer, C.[2]; Romp, E.[2]; Temml ,V.[1]; Garscha, U.[2]; Schuster, D.[1,3]; Werz, O.[2]; Matuszczak, B.[1]
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Currently, the main target for anti-inflammatory drugs is the cyclooxygenase (COX) pathway within the arachidonic acid (AA) cascade. COX inhibitors are established standard for treatment of acute inflammatory syndromes, but long term use causes severe gastrointestinal and cardiovascular side effects. Therefore, the other two pathways of the AA cascade have gained importance in the drug development, especially for the treatment of chronic inflammatory diseases like rheumatic conditions, systemic lupus erythematosus, multiple sclerosis and syndromes with chronic inflammation contributing to disease progression like Morbus Alzheimer and Morbus Parkinson. Recently, diflapolin, the first dual inhibitor of 5-lipoxygenase activating protein (FLAP) and soluble epoxide hydrolase (sEH) has been

discovered in a pharmacophore-based virtual screening approach. This compound leads to a decreased formation of pro-inflammatory leukotrienes (LTs) and simultaneously to an increased concentration of anti-inflammatory epoxyeicosatrienoic acids (EETs) by avoiding their sEH catalyzed degradation. It is a useful lead compound to investigate the biology of sEH and FLAP and their potential in treating inflammatory diseases. However, a disadvantage of diflapolin is the low water solubility. Generally, a low solubility causes a low and variable oral bioavailability and is subsequently detrimental for pharmaceutical use. Therefore, structural optimizations, which require knowledge of structure-activity relationships, are needed to create a promising drug candidate in the future.

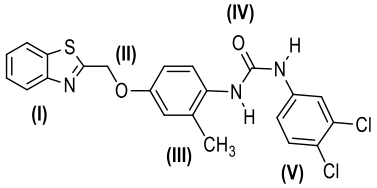

Figure 1. Structure of the first dual sEH/ FLAP inhibitor diflapolin

Diflapolin can be divided into five parts, the heteroaromatic core **(I)**, the spacer unit **(II)**, the methyl substituted para-phenylene **(III)**, the urea moiety **(IV)**, and the 3,4-dichlorophenyl substituent **(V)**. So far, we have performed modifications of the subunits **I**, **III**, **IV**, and **V**. The series of novel compounds were screened for FLAP and sEH inhibition. The results obtained indicated that modifications of all these subunits influence the biological activities. Here we will present multistep synthesis of these novel diflapolin derivatives, their biological activities, structure-activity relationships as well as results of molecular modelling studies.

Acknowledgement: Vieider, L. and Schoenthaler, M. would like to acknowledge support by the University of Innsbruck ('Doktoratsstipendium aus der Nachwuchsförderung')

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POS.38

Development of a Novel 3D Shape and Surface-Property Descriptor

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The representation of molecules for computational methods gets more and more sophisticated, starting with descriptors using simple physicochemical properties and leading up to representations employing the 3D geometry of a molecule. A challenge faced by all methods encoding the 3D information of molecules is conformational flexibility. The standard approach is to create a relatively large ensemble of conformers per molecule and use it for the intended study. The downside of the ensemble method is the computational cost required to generate appropriate conformers as well as the exponentially rising amount of calculations for encoding and numerically processing the conformer ensembles for machine learning. However, the ability of a bioactive molecule to interact with its target strongly depends on its 3D shape and the spatial localization of structural elements whose properties should be complementary to the target's binding site.
Therefore, to obtain a novel descriptor that takes these two critical factors into account, the Local Diameter Function [1] is adapted to molecules, yielding a translationally and rotationally invariant representation of a compound's 3D shape, which is robust to conformational changes. The conformation-robustness of the novel 3D descriptor allows the reduction of ensemble size by an order of magnitude, which considerably lowers computational cost.
Starting with a triangulated mesh of the molecular surface, the molecule's diameter in the neighbourhood of each vertex is calculated using a cone-shaped bundle of rays shot through the molecule. By recording the length of each ray in each bundle and statistically evaluating these lengths by bundle, numerical transformations yield a shape representation. Additionally, RDKit's Chemical Features [2] (i.e. H-bond donor/acceptor, aromaticity, pos./neg. ionizability, Zn-binding ability) and Local Hydrophobicity [3] are mapped onto the solvent excluded surface. These local surface properties hit by a cone-bundle's origin and base are recorded along with the Local Diameter and merged with the shape representation into the novel descriptor combining molecular shape and surface properties.
The development of the novel descriptor included systematic optimization of the most critical hyperparameters. The descriptor's robustness towards conformational change is evaluated on benchmark datasets of diverse molecular flexibility.

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POS.39

Lipid solubility prediction for drugs and drug-like molecules

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Solubility is an important thermodynamic property and plays a crucial role in drug discovery. Today many new highly potent drug candidates as outcomes from drug discovery programs are poorly or rather non-soluble in water. Consequently the oral bioavailability of these substances, which depends on the solubility and the dissolution rate in the gastrointestinal tract as well as on the membrane permeability, is low [1]. Hence, new formulation approaches are needed and one of the most investigated approaches are lipid-based formulations [1, 2]. These formulations are composed of different ingredients and finding the optimal composition can be very costly and time consuming. For these reasons computational tools predicting the solubility are needed to support the development of new formulations. There are many well-established methods, which address the water solubility, but only few attempts have been made to predict the solubility in single lipid excipients, let alone in lipid-based formulations [3]. Because of the complex nature of lipid-based formulations direct prediction is very challenging. Several approaches predict the solubility in each single excipient and use a mathematical combination to calculate the solubility in the formulation with some success [4, 5]. The main part of these formulations is lipids or mixtures of lipids. Therefore a reliable prediction in these solvents is crucial.
Quantitative Structure-Property Relationship models (QSPR) are a well-established method to link calculated properties of a substance, also known as descriptors, to the experimental property of interest (here: the lipid solubility) using a mathematical function. Choosing the set of descriptors is a crucial part to achieve a reliable mathematical model. It can be shown that descriptors solely derived from the molecular structure are not sufficient to predict the lipid solubility. Experimental descriptors, like the melting point or the heat of fusion, are needed to get a reliable result [5]. Only few values for lipid solubility that can be used for predictive modelling are published so far [5]. For this reason an expansion of the experimentally determined data base is urgently needed and will be presented in a separate contribution by the second author.
In this work, QSPR models with different descriptor sets are developed and compared towards their ability to predict the solubility in single lipid excipients. The descriptor sets are composed of a selection of descriptors calculated with standard software tools, properties derived from COSMO-RS [6], thermodynamic information from molecular dynamics simulations and experimental melting properties of the substance. The major goal is to evaluate the potential of molecular dynamics simulations, since it could be shown recently that a combination of molecular dynamics simulations and standard fingerprints

improve the prediction of water solubility and partition coefficients as compared to well-established methods [7].

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POS.40

A cyclic sulfamide as neutral solubility improving group in a new class of Aurora kinase inhibitors

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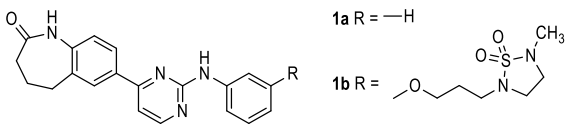
Aurora kinases are overexpressed in ovarian, prostate or colon carcinoma and constitute an attractive target in cancer treatment. Besides their regulatory function of mitotic processes, e.g. spindle formation during metaphase, they are involved in chromosome condensation, centrosome cycle and cytokinesis. The isoforms Aurora A, B and C are distinguished by expression level, localization and cellular activity [1,2]. Previous studies have shown that the new class of anilinopyrimidines represented by **1a** are Aurora A kinase inhibitors with single digit micromolar IC_{50}-values [3]. One of the problems frequently encountered with protein kinase inhibitors is poor solubility, which hampers a straightforward preclinical development [3]. To optimize the aqueous solubility, a cyclic sulfamide was introduced as neutral solubility improving group (NeuSIG). Although adding molecular weight and molecular complexity, NeuSIGs are moieties that are not increasing lipophilicity, hydrogen donator counts or stereocenters to the structure. Indeed, **1b** showed improved aqueous solubility with retained biological activity. The attachment point for the NeuSIG was selected based on docking studies and the results of X-ray structure analyses. An unexpected positive contribution of the NeuSIG was the improvement of selectivity for Aurora isoforms versus other protein kinases.



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POS.41

Towards New and Efficient Syntheses of Amino Alcohols and Diamines

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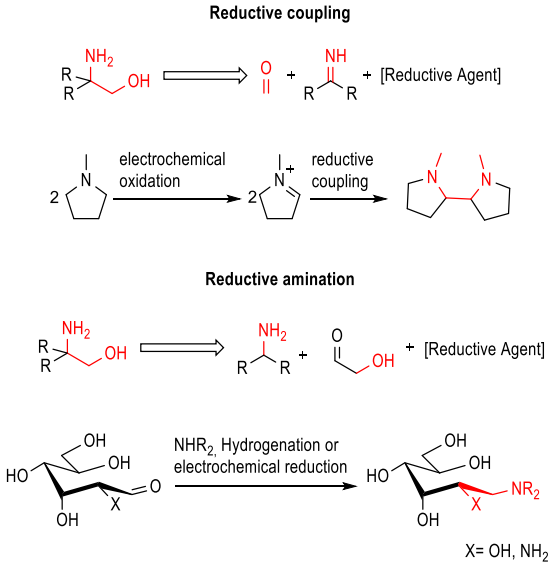
Small vicinal amino alcohols like 2-Amino-2-methyl-1-propanol (AMP) and 2-Amino-2-ethyl-1,3-propandiol (AEPD) are a class of industrially produced molecules with a broad field of application. They are alkaline (pK_a: 9-10), nontoxic, soluble in water, have a high chemical stability and a low vapor pressure at room temperature. Due to their favorable characteristics they are used as additives in cosmetics and pharmaceuticals. According to the European Chemical Agency (ECHA), these molecules are used up to a 10000 ton/a scale in Europe.[1] Compounds like AMP and AEPD are produced from small alkanes *via* a risky and energetically unfavorable route. Therefore greener and more economical synthetic approaches to these molecules are of great interest. Moreover, the synthesis of alternative derivatives, especially diamines with a higher atom economy is highly attractive. Our approach to achieve these goals is based on two different classes of reaction types: The first is the reductive coupling of imine derivates with carbonyl compounds, which is a direct approach to the already existing amino alcohols. This coupling uses readily available and cheap substrates and mild reaction conditions. Further on, green and cost-efficient reductive agents like magnesium or electrochemical pathways can be used.[2,3] The second reaction type is the reductive amination, where amines and hydroxy carbonyls (preferably from regenerative sources) can be used as starting materials.[4]



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POS.42

Determination of the solubility of poorly water-soluble drugs in different lipid-based single excipients and lipid-based formulations (LBFs)

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Throughout the process of drug development one obstacle to overcome for a majority of novel substances is their poor solubility in water. A major problem is the limited absorption in the gastrointestinal tract. In many cases this is detected late in the development process [1]. Due to the increasing number of water-insoluble and poorly-soluble compounds the focus shifted to finding alternative sophisticated formulations to enhance their bioavailability.
One approach is the development of lipid-based formulations (LBFs). These are complex systems which vary in the composition of oils, surfactants and co-solvents as well as in their proportions. A systematic development of lipid-based dosage forms is thus non-trivial due to this complexity.
The work presented here deals with method development for experimental determination of the solubility for selected compounds in lipid-based single excipients. For the time being no comprehensive protocol for this type of solubility determination has been published. Compounds with structural diversity and set parameter logP > 2 were studied. The substances were incubated and shaken for a defined time to dissolve in the excipients and then quantified by high performance liquid chromatography (HPLC). For the development of HPLC methods the aim was to test as many compounds as possible with the same column and mobile phase. The results were compared to Persson et al. and solubilities in many cases are well reproducible while in certain instances larger deviations could be observed.
In addition to the solubility measurements, the melting point (T_m) and the heat of fusion (ΔH_f) were examined via Simultaneous Thermal Analysis (STA), a combination of Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis. Accuracy and precision of the T_m and ΔS_f are of particular importance since they are the most important predictors for in-silico models to predict solubilities which are presented in a separate contribution by the second author. While the melting points are comparable to those found by Alskär et al. [2] and values given in literature [3,4] the calculated entropy of fusion (ΔS_f) shows a systematic shift obviously induced by the measured ΔH_f values. Though the origin of these deviations still could not be fully clarified, slight differences in the manually set set-points for the integration and the slope of the baseline of the heat release curves from the STA signals can be one reason. The determination of the origin of these deviations will be subject to a further study.

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POS.43

Limited proteolysis to reveal polymer surface interactions of site-selective PEGylated interferon-α2a variants

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Conjugating PEG to improve the serum half-life of biologics is a well-known and accepted technique, which has been used for various FDA approved drugs, including Pegasys® (PEGylated IFNα2a, Roche) PEGintron (IFNα2b, MSD) and ONCapsar (PEGylated Aspargase, Shire) [1-3]. As potential drawback, PEGylation goes in hand with a loss of biological activity, when unspecific conjugation chemistries (like NHS groups) for attachment of the PEG polymer are applied [4,5]. This limitation may be overcome by the usage of the amber codon suppression technique that allows the incorporation of unnatural amino acids (uAAs), which are suitable for strain promoted azide alkyne cycloaddition chemistry (SPAAC), yielding in site selective PEGylated products [6,7].

In this study, amber codon suppression was used to design site-selective PEGylated interferon-α2a (IFNα2a) variants, which were PEGylated with a 10 kDa PEG chain at position #31 or #134, respectively. The modified interferons were used to investigate the PEG to protein surface interaction compared to the wildtype protein deploying a limited proteolysis assay (LiP, Figure 1) [8]. The overall aim was to correlate biological potencies with the PEG to protein surface interaction at the molecular level to develop blueprints for the future design of fully active PEGylated IFNα2a proteins.

PEGylated IFNα2a variants were unspecifically digested for 1 min by proteinase K at an enzyme to substrate ratio of 1 to 30 at 37°C. Enzyme activity was afterwards stopped by adding the denaturating agent sodiumdeoxycholate to a final concentration of 5 % (w/v) and samples were heated for 3 min at 98°C. The partially digested sample was quantitatively digested with trypsin to produce LC-MS/MS compatible peptide fragments. By comparing the arising peptide fragments and their intensities with the appropriate control samples (IFNα2a WT and IFNα2a $K31N_3$) we determined the interaction sites of the PEG polymer with the protein surface.

The PEG-polymer conjugated to the position #31 was shown to shield residues #23, #24, #28-32 and #76 of the conjugate as analysed by peptide fragment intensity ratios compared to the wild-type Interferon-α2a protein and its unconjugated mutant, respectively. These data are currently combined with the analysis of PEGylated IFNα2a at position #134 to reveal other potential interaction sites of the hydrophilic polymer.

In conclusion, LIP is a suitable method to screen polymer-protein interactions at the molecular level with potential to rationally design fully active PEGylated protein conjugates in the future.

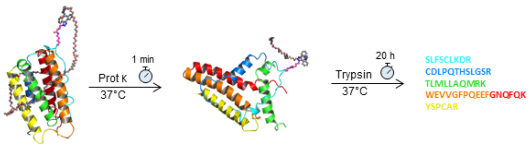

Figure 1: Schematic of the used LIP assay as performed with site-specifically PEGylated Interferon-α2a.

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POS.44

Synthesis of Dithiocarbamate-Derivatives and their *in vitro* testing against *Schistosoma mansoni* as Novel Anthelmintic Inhibitors

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Schistosomiasis, also known as bilharzia, is a chronic parasitic disease infecting more than 250 million people. At least 200,000 dead persons are associated with the disease. Beyond Malaria, it is the second most important parasitic disease worldwide occurring in over 70 countries in tropical and subtropical regions [1]. But there is recent evidence of an autochthonous site of infection in Southern Europe (Corsica) [2]. The schistosome species are transmitted by contact of water containing free-living larval forms of the parasite (cercariae) originating from intermediate host snails. Due to the lack of a vaccine, the therapy is restricted to a single drug. Praziquantel is the only drug effective against all schistosome species and has been used for more than 40 years. Every year, millions of people are medical attendance with praziquantel. Therefore, the fear of drug resistance encourages the search for novel anthelmintic drugs against schistosomiasis [3-6].

Dithiocarbamates were identified as anthelmintic compounds from a screening with disulfiram. Disulfiram was used for the treatment of chronic alcoholism by inhibiting the acetaldehyde dehydrogenase converting acetaldehyde to acetic acid. It was recognized that disulfiram also disintegrates the surface structure of schistosomes, the tegument, leading to the death of the parasites. We synthesized dithiocarbamates instead of dithiocarbamate disulfides. Our dithiocarbamate compounds show significant effects on adult schistosomes *in vitro*. Possibly, this discovery might open a new direction for chemotherapy of human schistosomiasis.

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POS.45

Synthesis of fluorine-containing PDE10A-Inhibitors as potential Ligands for Positron Emission Tomography (PET)

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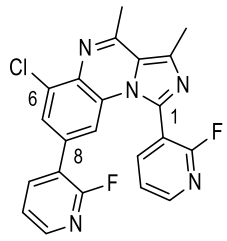
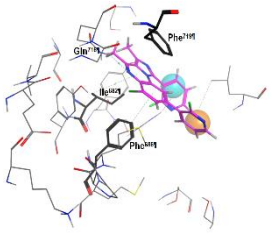
Phosphodiesterases (PDE) are hydrolysis enzymes of secondary messengers and therefore important regulators of signal transduction. PDE10A, a cAMP- and cGMP-sensitive hydrolase is primarily expressed within the striatum and identified as a potential target for therapy and diagnosis of various disorders of the central nervous system (CNS) such as schizophrenia or Huntington's disease. Recently, it has been reported that 1-Arylimidazo[1,5-*a*]quinoxalines are potent and selective inhibitors of PDE10A and are suitable for imaging the enzyme in physiological and pathophysiological environments.
In this thesis, the development and synthesis of new inhibitors are presented, which should serve as reference compounds in view of their potential use for positron emission tomography.
After the successful adaptation of a 6-step synthesis, 6-chloro-imidazo[1,5-*a*]quinoxaline was prepared as basic structure and derivatized by *Suzuki* coupling. The obtained mono- and disubstituted pyridinyl derivatives and their precursors were elucidated by high-performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS). The pharmacological characterization of 12 obtained target compounds was done by determining the inhibitory effect against human PDE10A at 10nM, against PDE2A at 1µM. Based on this data, 6-chloro-1,8-bis-(2-fluoropyridin-3-yl)-3,4-dimethylimidazo[1,5-*a*]quinoxaline was selected for the determination of the IC50-values for PDE10A and PDE2A. The model of the S9 fraction was selected for the testing of the metabolic stability, the chemical stability was investigated under acidic and basic conditions. A docking study visualized possible binding modes of the inhibitors in the proteinogenic environment of PDE10A.





IC50(PDE10) = 0,307 nM
IC50(PDE2) = 57,7 nM

POS.46

Rational Design and Synthesis of 4-Acyl-Pyrrol-based Dual HDAC/BRD4 Inhibitors

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In recent years epigenetic modifications and their crucial role in regulation of gene expression have been discovered and widely studied. Many enzymes have been identified that modify the chromatin structure. For instance, via histone lysine acetylation and deacetylation epigenetic marks are either attached resulting in open, transcriptionally active chromatin or removed resulting in condensed, transcriptionally repressed chromatin. Deregulations of these mechanisms are strongly associated with the pathogenesis and progression of several human diseases. [1] Histone deacetylases (HDACs) remove acetyl groups on histone and non-histone proteins and hereby influence cell cycle progression, cell proliferation and differentiation. HDACs are over expressed in several cancer types and inhibition of HDAC function can result in various anti-cancer effects. [1] As a consequence, HDAC inhibitors (HDACi) have established themselves as anti-cancer drugs and four compounds (vorinostat, romidepsin, belinostat and panobinostat) have been FDA-approved for the treatment of T-cell lymphoma or multiple myeloma. [1] *N*-Acetyl-lysine marks are recognized by bromodomain containing proteins (BRDs) which have more recently emerged as epigenetic drug targets. [3] BRDs bind to acetylated lysine residues and recruit enzymes of histone modification, including histone acetyl transferases (HATs) and HDACs. This way BRDs influence cell proliferation and apoptosis, and deregulation is associated with the development of cancer. [2] BRD inhibitors (BRDi) have shown various anti-cancer effects including anti-proliferative activity against solid tumors and significant anti-leukemic effects. [3] Furthermore, combinations of established HDACi and BRDi have shown promising synergistic anti-proliferative effects against cancer cells. [4,5]

Given the synergistic effects of HDACi and BRDi and the advantages of polypharmacology e.g. a lower probability of developing target-based resistance and a more predictable pharmacokinetic profile compared to drug combinations, dual HDAC/BRD inhibitors could be a promising new class of multi-target dugs. [2,3]

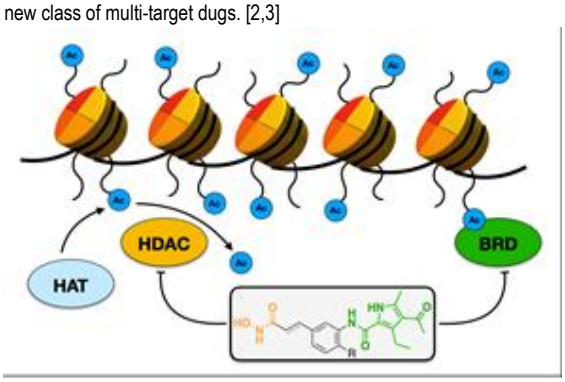

Figure 1: Acetylated chromatin, open and transcriptionally active. The dual HDAC/BRD inhibitor is able to interact with both target structures and efficiently disrupts acetyl lysine signaling.

Consequently, we herein present the design, synthesis and biological evaluation of a small library of new dual HDAC/BRD inhibitors based on the 4-acyl-pyrrol BRD4 inhibitor XD14 [6] and an established HDACi scaffold (Figure 1). [1] To synthesize the target compounds we first prepared acetoacetamide derivates starting from *Meldrum´s acid*. The

acetoacetamides were used as starting materials to obtain pyrrole α-amide analogs via *Knorr* synthesis. Finally, the zinc-binding group was introduced using a three-step protocol. The subsequent biological evaluation confirmed that some of the compounds are dual HDAC/BRD inhibitors with anti-proliferative properties. Thus, this series of compounds is a good basis to deepen our understanding of multi-target drugs and for the future development of dual HDAC/BRD inhibitors.

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POS.47

Design of light-activatable LpxC inhibitors

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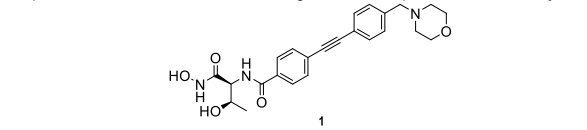
The excessive use of antibiotics in therapy and agriculture lead to the emission of these compounds into the environment. This is one reason for the increasing resistance of bacteria against antibiotics. The design of antibiotics that gain their biologic activity only after the irradiation with light is a promising approach to circumvent this problem.[1]
Photo-activatable antibiotics can be obtained by connecting literature-known inhibitors with photo leaving groups (PLGs). The irradiation with light causes the cleavage of the PLG, which results in the formation of radicals, carbenium ions[2] or carbenes[3] that can bind covalently to the to be inhibited enzyme.
The deacetylase LpxC, which participates in the biosynthesis of lipid A in gram-negative bacteria, is a validated antibacterial target. Most of the LpxC inhibitors described in the literature contain a chelator moiety that can coordinate the catalytic Zn^{2+}-ion and a lipophilic side chain addressing the hydrophobic tunnel of the enzyme.[4] Beside these features the potent inhibitor CHIR-090 **(1)** can form hydrogen bonds between its hydroxy group and conserved side chains of the enzyme.[5] The replacement of this hydroxy group by several photo leaving groups, leading e. g. to phenyl ethers **(2)** as well as oxadiazolines **(3)**, should enable the photochemical generation of carbocations inside the binding pocket. These cations can react with nearby nucleophilic side chains.
To improve the photo leaving groups and to optimize cation generation the photoreactions were further investigated via computational chemistry.



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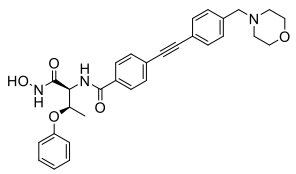

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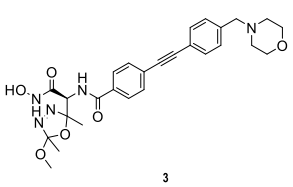

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POS.48

Targeting bacterial RNAseP

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The CDC declared 2013 that we are living in an “post antibiotic era” due to rapidly rising resistances. Cases of severe infections with gram-positive bacteria as *MRSA* are becoming more common and even more worrisome are infections with gram-negative bacteria like *P. aeruginosa* that put us on the edge of therapy options. [1] RNAseP is a highly conserved ribozyme throughout gram-positive and gram-negative bacteria. It consists of an RNA-part and a small proteine unit and is responsible for the processing of 5'-end tRNA. [2,3] Our aim is to inhibit this ribozyme by addressing its protein part using unsymmetrically substituted carboxylic acid diamides.

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POS.49

Studying Protein Protein Interactions in Human Protein Kinase CK2 by Click Chemistry and Photocrosslinking

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Human protein kinase CK2 is a heterotetrameric Ser/Thr kinase, consisting of two catalytic (CK2α or α') and two regulatory (CK2β) subunits. CK2 is a target for anti-cancer drugs, as it has been shown, that many different types of tumours are going along with an elevated level of CK2. Inhibition of CK2 to normal levels results in apoptosis of neoplastic cells [1]. Most of the CK2 inhibitors are addressing the ATP binding site, which is conserved among various kinases and hence leading to limited selectivity. Targeting the CK2α/CK2β subunit interface could lead to more selective inhibitors of this cancer target.
A prerequisite for developing such protein protein interaction inhibitors (iPPI) is a method to analyse and measure this interaction.
In this study click chemistry and photocrosslinking were applied to study the interactions in CK2. Therefore, the unnatural amino acid *p*-azidophenylalanine (pAzF) [2] was incorporated into CK2α. Performing the SPAAC click reaction (Strain-Promoted Alkyne-Azide Cycloaddition) [3] CK2α was site specific labelled with a dibenzylcyclooctyne-fluorophore (DBCO-fluorophore) [4]. Microscale thermophoresis (MST) analysis with the fluorescently labelled CK2α and CK2β enabled the determination of their binding affinity.
Furthermore, pAzF was incorporated into CK2β at different positions. Among them positions at the CK2α/CK2β interface as well as a position far from this site. The successful incorporation of pAzF was proved by CuAAC click reaction (Copper(I)-Catalyzed Alkyne-Azide Cycloaddition (CuAAC) with a fluorophore. Capillary electrophoresis analysis showed the ability of the modified CK2β variants to bind to CK2α leading to an increase of its catalytic activity. The interaction of the modified CK2β variants with CK2α was further analysed by photocrosslinking. Therefore the CK2β variants were incubated with CK2α, irradiated by UV light of 365nm and analysed by Western Blot.

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POS.50

Fighting the scourges of mankind – Targeting eIF4A and RNAseP

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From April to June 2018 60 new confirmed cases of *Ebola* with 28 deaths occurred in the Democratic Republic of Kongo. [1] The most severe outbreak of this haemorrhagic fever was from April 2014 to 2016 with 28652 suspected and confirmed cases and 11325 deaths. [2] The virus spread rapidly and - as the numbers show – is highly lethal. To the day therapy options are limited. Recently Grünweller *et al.* found a natural compound that showed activity against *Ebola* in infected cells. To synthesise drug like compounds we used a computer assisted approach to simplify the structures and ended up synthesising hydantoines and triazines.
Not only *Ebola* is a threat to mankind but also emerging resistances against antibiotics. Massive use of antibiotics in livestock, overuse and wrong prescriptions in medicine accelerate resistance crisis. The resistances are spread through the spectrum of bacteria, infections with gram-positive bacteria like *MRSA* as well as gram-negative bacteria like *P. aeruginosa* are becoming a common threat. [3] RNAseP seems to be a promising target as it is in gram-positive as well as in gram-negative bacteria existing. It is responsible for the processing of the 5'-end tRNA. As a ribozyme it consists of a RNA unit and a protein part. [4,5] On the basis of the protein unit's structure we synthesised drug like compounds.

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POS.51

Application of virtual screening, homology modeling, and molecular dynamics simulations to design isoform selective HDAC11 inhibitors

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HDAC activity is up-regulated in many types of cancer and HDAC inhibitors (HDACi) are successfully used as drugs for the treatment of cancer. So far the approved HDACi are all non-selective inhibitors and therefore showing cross-reactivity, toxicity, and at the end unwanted side effects [1]. To address this issue we focus on the application of computer- and structure-based approaches to come up with novel isoform selective HDAC8, HDAC10 and HDAC11 inhibitors.

In the current study we put emphasize on the most recently identified zinc-dependent HDAC family member HDAC11. Depletion of this enzyme showed good apoptotic effects on cancer cell lines, whereas no significant detectable effects on different types of normal cells were observed [2]. Potentially HDAC11-selective inhibitors could not only find application in the field of oncology, but also for the treatment of inflammatory and immunological diseases, since it has been reported to regulate various immune cells [3]. Due to the absence of a crystal structure for HDAC11, a homology model (HM) was generated. 3D-structures of hHDAC6 and hHDAC8 were chosen as multiple templates for the generation of the HM. Subsequently, molecular dynamics simulations were carried out to verify the stability of the generated model and molecular docking studies were applied on a recently published novel series of inhibitors to confirm its applicability for further studies. Finally, several target based virtual screenings were carried out to identify first small molecule inhibitors of HDAC11 [4]. In addition, structural differences at the binding domain between HDAC6, 8, 10 and 11 were analyzed to facilitate and assist the further drug design process.

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POS.52

Antitumor effect and potentiation of cytotoxic drug activity of a dual topoisomerase inhibitor on ovarian cancer

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Introduction: Ovarian cancer constitutes one of the most lethal of all gynaecologic neoplasms [1,2]. Late diagnosis and insufficient treatment options are responsible for cancer deaths. Although these options have improved during the last years, ovarian cancer is still not sufficiently treatable due to chemotherapy resistance and due to treatment limitations by side effects. Hence, research on new drugs for ovarian cancer therapy such as P8-D6 with promising antitumor properties is existential. P8-D6 is an effective inductor of apoptosis by acting as a dual topoisomerase inhibitor. Its outstanding proliferation inhibition was shown in the NCI-60 DTP human tumor cell line screening (GI_{50}/60 value: 49 nM) and in further experiments with ovarian cancer cells [3,4].
Methods: To investigate the viability and apoptosis of established ovarian cancer cell lines and ex vivo primary cells after treatment with P8-D6, the cells were analysed by using ApoLive-GloTM Multiplex Assay (Promega) and flow cytometry. For better comparison, cells were treated with different concentrations of P8-D6 and standard therapeutics for 48h. Likewise, the effects on the non-cancer human ovarian surface epithelial cells were determined by same protocol. Previous dual topoisomerase inhibitors like intoplicine were tested in clinical trials, but stopped due to hepatotoxicity. So the oxidative stress and induction of apoptosis after treatment with P8-D6, PBS or Doxorubicin for 48h were tested in human hepatocytes. The expression level of topoisomerase I or II could be a marker in P8-D6 therapy. So the levels of the tested cells were measured by western blot.
Results: In the ApoLive-GloTM Multiplex Assay results a significantly increase of apoptosis by P8-D6 than its references could be determined. These results could not only be detected in established cell line cells but also in primary patient cells. A considerably antiproliferative effect in tumor cells was observed in nM range as determined by IC50 calculation. Moreover, flow cytometry analyses showed a strong and rapid induction of apoptosis by P8-D6. Non-cancer cells were slightly effected by P8-D6. Also no hepatotoxic effect in *in vitro* studies on human hepatocytes was seen compared to PBS control.
Conclusion: P8-D6 has promising antitumor properties in *in vitro* studies on ovarian cancer. It has fewer side effects on normal ovarian cells than references and no hepatotoxic effect on human hepatocytes. Furthermore P8-D6 should be tested in 3D culture and in an ovarian cancer xenograft mouse model as an *in vivo* study to determine the therapeutic efficiency.

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POS.53

Structure-Based Virtual Screening for Novel Potential Inhibitors of the Dual Leucine Zipper Kinase (DLK)

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The Dual Leucine Zipper Kinase (DLK) is expressed in the peripheral and central nervous system being required for neurodegeneration and axon regeneration, and in the insulin-producing beta cells. The DLK, also known as MAP3K12, is integrated in different MAPK signaling pathways activating downstream kinases like p38 and JNK. JNK and DLK inhibit the important transcription factors MafA and CREB respectively, which are necessary for insuline-gene transcription and secretion. An overexpression of DLK contributes to a loss of beta-cell function and inferentially to diabetes mellitus.[1]
Some ATP competitive small molecule inhibitors of the ATP binding site of the DLK, which are structurally based on the same principle, are already known from literature (Figure 1).[2] In our project, we strive for finding diverse potential inhibitors with novel scaffolds with the use of structure-based virtual screening (Schrödinger Maestro[3]).
For this purpose, we chose the Clean-Druglike subset of the ZINC database[4], which contains 5.1 mio. compounds fulfilling inter alia the "Lipinski's Rule of Five". Additional filter criteria were applied (e.g. number of rotatable bonds, number of heteroatoms) and 3.8 million compounds were left to be screened. Known active DLK inhibitors were added to the molecule library to investigate the quality of docking and scoring during the screening process.
Tautomers and protomers were generated using Schrödinger Maestro. Four DLK X-ray structures of the Protein Data Bank[5] were further analysed by Molecular Dynamics Simulations and prepared for molecular docking. Testdockings were carried out with a small ligandset of known actives and decoys to reduce the amount of targets in favor of a high capability of enrichment for the ZINC screening. Finally, six DLK conformations were chosen as targets for the Virtual Screening Workflow.
HTvS (*High Throughput virtual Screening*) was carried out with at least two forced hydrogen bonds to the hinge region of the ATP binding site. After additional SP (*Standard Precision*) screening, about 800,000 compounds were ranked by their dockingscore. Taking the Top 5% of each target and calculating a consensus score using different scoring methods (dockingscore, MM-GBSA, HYDE[6], QSAR) to optimize the enrichment, nearly 124,000 compounds were further considered for a clustering to reduce the size of dataset in favor of structurally diverse molecules. After visual inspection of the reduced database, molecules are chosen to be tested for their DLK inhibiting property by ADP GLO Kinase Assay.[7]

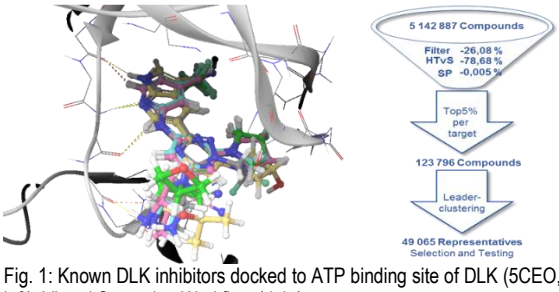

Fig. 1: Known DLK inhibitors docked to ATP binding site of DLK (5CEO, left); Virtual Screening Workflow (right)

References:
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POS.54

Synthesis and *in Vitro* Testing of Dithiocarbazate Derivatives as Potential Anthelminthic Agents against *Schistosoma mansoni*

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Schistosomiasis is one of the most important parasitic infections worldwide and the treatment of the trematode triggered disease is currently almost completely dependent on Praziquantel. Due to the widespread and decades-long application, the concern about resistance development is great, so that there is a need for new antischistosomal active substances. [1,2]
In previous studies of our group dithiocarbamates displayed antischistosomal activity, hence we also investigated the structurally related dithiocarbazates. After successful synthesis we examined the antischistosomal activity by means of *in vitro* tests on *Schistosoma mansoni*. A number of initial derivatives exhibited activity against the parasites, providing another interesting starting point for the development of novel antischistosomal compounds. Subsequently, the toxicity of the active compounds to human cells was evaluated (HepG2, LS174T) and a certain cytotoxicity was observed, thus this aspect should also be considered in future optimisations.

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POS.55

Development of fluorescent probes for monitoring 5'-*ecto*-nucleotidase (CD73) – a biomarker for cancer cells

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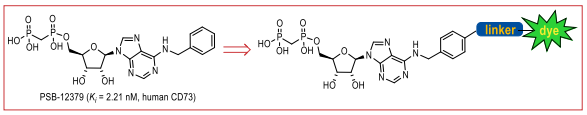
Ecto-5'-nucleotidase (CD73) is a member of the *ecto*-nucleotidase family, which catalyzes the dephosphorylation of extracellular nucleotides. CD73 catalyzes the hydrolysis of nucleoside monophosphates, mainly of AMP, producing the signaling molecule adenosine. Further *ecto*-nucleotidases include the nucleoside triphosphate diphosphohydrolases (NTPDases; subtypes 1, 2, 3 and 8), the nucleotide pyrophosphatase/phosphodiesterases (NPP1-4) and the alkaline phosphatases (APs).[1] CD73 is often co-localized with adenosine receptors, and CD73 inhibitors reduce extracellular adenosine levels, which results in an indirect blockade of adenosine (P1) receptor activation. Many tumor cells were found to overexpress *ecto*-nucleotidases, which metabolize pro-inflammatory ATP into anti-inflammatory, immunosuppressive, tumor growth-stimulating, angiogenic adenosine.[2] Therefore, *ecto*-nucleotidases possess potential as novel drugs, e.g. for cancer (immuno)therapy or for the treatment of neurodegenerative diseases.
To monitor the expression level of CD73, a fluorescent marker molecule with a high binding affinity, that can be used instead of an antibody, is highly desirable. The ADP analogue α,β-methylene-ADP (AOPCP, K_i = 88.4 nM, human CD73) was the first described potent competitive inhibitor of CD73.[3,4] Since its discovery, significantly more potent AOPCP-based inhibitors have been developed by our group that display high selectivity and metabolic stability.[4] One of these compounds, PSB-12379, was selected as a lead structure to develop potent fluorescent marker molecules with high binding affinity. The idea was to attach a fluorescent dye to the benzyl ring in the N^6-position via a linker moiety. To optimize the binding properties, multiple factors have to be taken into account, including the linker length, the lipophilicity of the linker, and the connection between the linker and the structure of the small molecule

inhibitor. The obtained fluorescent CD73 inhibitors will be useful tools for research and diagnostic applications.



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POS.56

Sirtuin inhibition employing the specific interaction with the conserved Arg97 residue

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Sirtuins belong to class III of the histone deacetylases (HDACs) [1] and are able to cleave off several different acyl groups from the ε-nitrogen of lysine residues. Unlike other HDACs the sirtuins are not Zn-dependent but use NAD+ as a cofactor for deacylation. [2] There are seven human sirtuin isotypes that differ in their cellular localization, catalytic activity and their acyl substrates. [3] The great range of different substrates makes them crucial for various cellular processes like metabolism, DNA repair, mitosis, stress response or cell survival and apoptosis. [4] Therefore dysregulation of sirtuins is associated with the pathogenesis of various diseases like cancer, neurodegenerative diseases (Alzheimer's or Huntington's disease) or metabolic diseases (type II diabetes). [5] To further investigate the functions of the sirtuins chemical probes and modulators are needed.

Based on the structure of the sirtuin rearranging ligands (SirReals) we designed chemical probes for Sirt2 using click chemistry. Therefore a SirReal-head is linked to a specific tag (e.g. fluorescence/affinity tag) through a triazole moiety. Crystal structures show that this triazole actively contributes to Sirt2-binding by a specific interaction with Arg97. [6] Since this arginine residue is conserved in all human sirtuins, we hypothesized that other isotypes could be reached by removing the 4,6-dimethyl¬mercapto¬pyrimidine moiety that is crucial for Sirt2 binding. Indeed a shift of inhibition from Sirt2 towards Sirt1 for this mini-SirReal was observed. This is a promising first step towards new inhibitors of other sirtuin isotypes based on the SirReal structure.

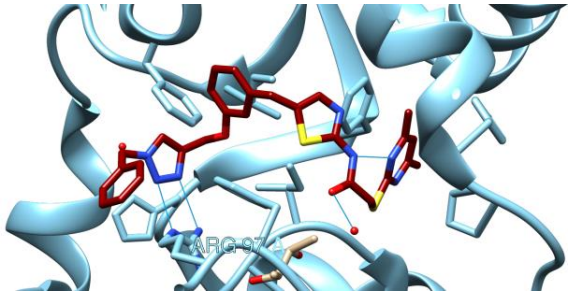

Figure 1. Crystal structure of Sirt2, showing the interaction between the triazole moiety and Arg97.

We thank the DFG (Ju295/14-1) for funding.

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POS.57

Structure-based design and synthesis of novel hydroxamates as dual histone deacetylases/ bromodomains epigenetic inhibitors for anti-parasitic therapy

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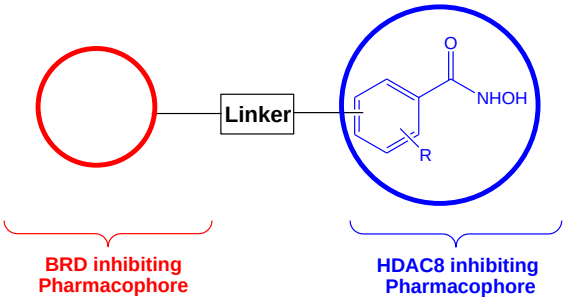
Human parasitic infections are among the major and most serious health problems, especially in the underdeveloped regions of the world. Limited number of available drugs, side effects and rapid development of resistance increase the severity of these neglected diseases, and necessitate the discovery of new therapeutics with novel mechanisms of action [1]. In this regard, the "repurposing or piggy back" approach represents an attractive and promising strategy. If human cells –such as cancer- and parasites have closely related and validated targets, then it is very probable that known anticancer inhibitors of those targets can also inhibit the parasites. In the field of epigenetics, this approach was found to be very effective, as many epigenetic targets –mainly histone modifying proteins- were found to control gene expression in major human parasites and inhibitors of these targets were designed and optimized to inhibit different types of parasites [1-6].
Histone acetylation is one of the most studied epigenetic regulation mechanisms. Histone modifying proteins include writers; Histone acetyltransferases (HATs), readers; Bromodomains and erasers; Histone deacetylase (HDACs). Alteration in balance between HATs and HDACs are related to many diseases such as cancer [7]. HDACs and bromodomains were identified as potential therapeutic targets for cancer therapy [7] and combination of their inhibitors showed a remarkable activity against murine lymphoma [8]. Some studies reported the design and synthesis of some dual inhibitors of both targets and their activity against some cancer cell lines [9-12]. This proof of evidence of the significance of inhibiting both targets in cancer encouraged us to apply the "piggy back" strategy to design novel dual acting agents that can inhibit both targets in parasites.
Very recently we identified several bromodomain inhibitors active against parasites [13]. In the current study we focused on aromatic hydroxamic acids as specific smHDAC8 inhibitors and coupled them to several specific bromodomain-inhibiting scaffolds. The HDAC8 inhibitor scaffolds were formerly identified as promising anti-parasitic compounds [14].



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POS.58

Targeting Protein-Protein Interactions: Assays for Methyl-Lysine Reader Proteins and Inhibitor Development

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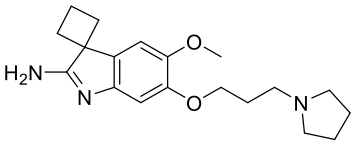
Epigenetic marks established on histone tails by specific enzymes play an important role in gene regulation. Methylation of lysine of histone tails can be related to both activation and silencing of genomic information. Specific reader proteins for these marks are therefore crucial to mediate the right cellular effect, which makes them interesting drug targets especially as they often are dysregulated in different cancers and other diseases. Spindlin1 is a methyl-lysine reader specific for trimethylation of lysine 4 of histone H3 (H3K4me3). By recognizing this mark, it is activating the Wnt signaling pathway. It recently was shown to be overexpressed in several cancers [1]. SUV39H1 is a multidomain protein including a SET domain catalyzing trimethylation of lysine 9 on histone H3 (H3K9me3), a mark associated with gene silencing. By an additional reader domain, it also recognizes its own mark, leading to amplification of the silencing mark. SUV39H1 plays a role in different diseases [2].
We built an assay platform to screen for inhibitors of methyl-lysine reader proteins, including biochemical (AlphaLISA®, Fluorescence Polarization) and biophysical (Thermal Shift, Bio Layer Interferometry) methods. This platform, which was first established for Spindlin1 [3] and later adapted to SUV39H1, allows initial as well as orthogonal counter-screening of candidate compounds. As the components are universally adaptable to any protein-protein interaction, this platform is a universal approach for screening for inhibitors of these interactions in-vitro. Further interaction partners are currently being adapted to this assay platform.
For Spindlin1, initial screening of a focused library lead to the discovery of A366, a recently published selective probe of histone methyl transferase G9a (IC50: 3.3 nM). It shows high affinity towards Spindlin1, resulting in IC50 of 186 nM. As the compound is not selective for this interaction, further chemical optimizations were carried out in order to gain selectivity against G9a, while maintaining affinity towards Spindlin1.



A366
IC_{50} Spindlin1: 182 nM

Figure 1: (left) Structure of the Spindlin1 inhibitor A366, (right) representative inhibition curve

We thank DFG (SFB992-MEDEP) for funding.

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POS.59

Identification of ecto-5'-nucleotidase (CD73) inhibitors by high-throughput screening – new potential drugs for cancer immunotherapy

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Extracellular ATP acts as a proinflammatory signaling molecules via G protein-coupled P2Y receptors and ATP-gated ion channels (P2X receptors).[1,2] It is hydrolyzed by a family of ecto-nucleotidases: in a first step ecto-nucleoside triphosphate diphosphohydrolases (NTPDases, CD39) and nucleotide pyrophosphatases/phosphodiesterases (NPPs, CD203a) convert ATP to AMP, which is then further hydrolyzed by ecto-5'-nucleotidase (CD73) to adenosine. Adenosine activates G protein-coupled adenosine (P1) receptors; it exerts powerful immunosuppressive properties via A_{2A} and A_{2B} adenosine receptor activation.[3,4] The well-balanced system of pro-inflammatory ATP and immunosuppressive adenosine is disturbed under several pathological conditions. Many tumors overexpress ecto-nucleotidases which leads to high levels of adenosine in the tumor microenvironment resulting in tumor immune escape.[5] Thus, inhibition of ecto-nucleotidases has been proposed as a novel strategy in cancer immunotherapy.[6] The present study was aimed at identifying novel scaffolds for CD73 inhibitors. Therefore, we established a CD73 assay suitable for high-throughput screening based on the detection of phosphate by malachite green. We adapted the assay to a robotic screening platform and initially screened a purine target-focused library of 6.000 small molecules. A primary screen resulted in 85 hit compounds (hit rate: 1.4%). For hit validation, a previously developed sensitive radioassay was employed,[7] which led to the confirmation of 27% of the hit compounds. Several new scaffolds were identified, which inhibited the enzymatic activity by more than 50% at a concentration of 10 µM. Among these, dual CD73 inhibitors / adenosine A_{2A} receptor antagonists were identified that showed similar potency at both targets.

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POS.60

Design, Synthesis and Biological Testing of novel EGFR Inhibitors with Low Nanomolar Activity against the Osimertinib-Resistant L858R/T790M/C797S Mutant

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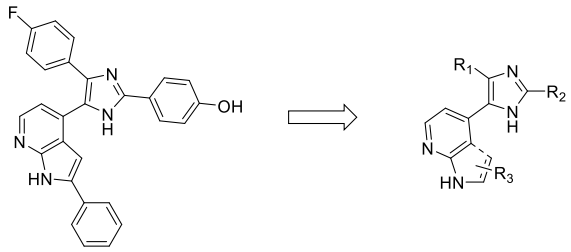
The treatment of non-small-cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) inhibitors is made challenging by acquired resistance caused by somatic mutations [1]. Third generation EGFR inhibitors (WZ4002, Osimertinib) have been designed to overcome resistance, mediated by the T790M mutation, through covalent binding to the Cys797 residue of the enzyme. These inhibitors are effective against most clinically relevant EGFR mutations, however their high dependence on this particular interaction means that additional mutation of Cys797 results in poor inhibitory activity, which leads to tumour relapse in initially responding patients [2,3].



Based on a selectivity screening of a highly potent reversible p38 inhibitor [4], we identified EGFR inhibition as an off-target effect of this compound. High potency, as well as moderate physicochemical properties and cellular activity against p38, led us to pick this compound as a lead structure for further improvements in terms of mutant EGFR inhibition. With this concept, we have successfully developed highly potent reversible and irreversible T790M EGFR inhibitors that showed picomolar IC_{50}-values in an enzyme assay and down to 14 nM EC_{50} in a double mutant (L858R/T790M) cellular assay. In contrast to classic third generation EGFR inhibitors, some of these compound showed high inhibitory activity in the low nanomolar range against the therapy-resistant L858R/T790M/C797S EGFR triple mutant [5-7].

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POS.61

Synthesis and *in vitro* characterisation of novel inhibitors of *Trypanosoma cruzi* histone deacetylase 2 (TcDAC2)

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The American trypanosomiasis, also known as Chagas disease, has been recognized by the WHO as one of the neglected tropical diseases and is estimated to affect 8 million people worldwide.[1] This disease is caused by the protozoan parasite *Trypanosoma cruzi,* which is transmitted by triatomine bugs. Endemic in Latin America Chagas disease has spread from its original boundaries through migration and so becoming a global issue.[2] Current treatment is limited to benznidazole and nifurtimox, which are associated with severe side effects, low cure rates in the chronic stage and the emergence of drug resistance.[3] Histone deacetylases (HDACs) are validated drug targets in cancer therapy and there is evidence that they can also be adressed to treat parasitic infections. In *T.cruzi* four zinc dependant HDACs has been identified. We focus on the *Trypanosoma cruzi* deacetylase 2 (TcDAC2), the homologous isoform of SmHDAC8 in *Schistosoma mansoni*, which was identified as a promising target for antiparasitic drug discovery.[4; 5] Recently, we tested several in house HDAC inhibitors on TcDAC2 *in vitro*. Aromatic hydroxamic acids were identified as promising hits that are able to kill the parasite in an *in vitro* assay. The synthesis and biological characterisation of novel TcDAC2 inhibitors is being carried out.

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POS.62

Argininamide-type neuropeptide Y (NPY) Y_1 and Y_2 receptor antagonists: SAR at the Y_1R and synthesis of potential molecular tools for the Y_2R

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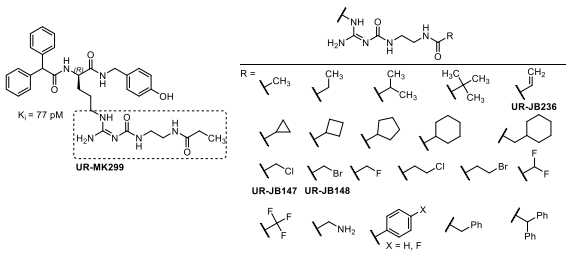
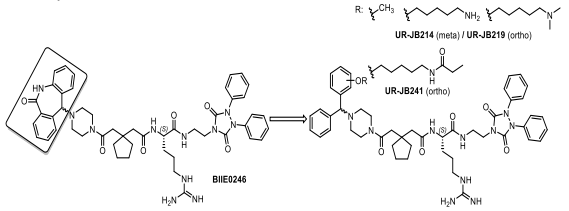
The N^{ω}-carbamoylated (R)-argininamide UR-MK299, a highly selective Y_1 receptor (Y_1R) antagonist with a K_i value < 100 pM, [1] exhibits approximately 20 times higher receptor affinity compared to the non-carbamoylated compound BIBP3226. The recently resolved crystal structure of the human Y_1 receptor (Y_1R) in complex with UR-MK299 [2] suggests that in the binding region of the propionamide moiety of UR-MK299 hydrophobic substituents may not only be tolerated, but should lead to an even higher affinity. Therefore, we replaced the propionyl moiety in UR-MK299 by various acyl substituents (acyclic and cyclic). There was an inverse correlation between Y_1R binding affinity and the bulk of the acyl moiety, which was especially pronounced in case of the cyclic substituents. The chloroacetyl derivative UR-JB147, showing the highest Y_1R affinity (K_i = 53 pM) in this compound library, as well as the bromoacetyl derivative UR-JB148 and the acryl derivative UR-JB236 represent potential molecular tools for a covalent labelling of the receptor.



Previously, a radiolabelled Nω-carbamoylated derivative of the (S)-argininamide BIIE0246 ([^{3}H]UR-PLN196) [3], was demonstrated to exhibit moderate Y_2R affinity (K_d 67 nM) and pseudoirreversible binding at the Y_2R. Amino-functionalization enables tritium-labeling, e.g., with commercially available succinimidyl [^{3}H]propionate, [^{3}H]methyl iodide or [^{3}H]methyl nosylate. The "cold" form (UR-PLN196) was an insurmountable antagonist upon stimulation of the Y_2R with pNPY. Aiming at a high affinity antagonistic Y_2R radioligand, in particular the boxed partial structure of BIIE0246 was varied.



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POS.63

Synthesis and SAR of desoxygenated *C*-furanosidic LpxC inhibitors

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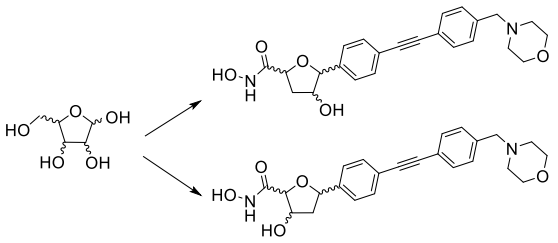
Due to the inadequate or too broad use of antibiotics in health care and agriculture as well as the discovery void of novel antibiotics over the past three decades, we encounter a significant rate of multi-drug-resistant (MDR) bacteria today. The latter cause severe nosocomial infections that are not responsive to common antibiotics anymore. To overcome bacterial resistance, there is an urgent need for new unprecedented bacterial targets. [1], [2]
LpxC, a Zn^{2+}-dependent deacetylase, suits perfectly as such a target in Gram-negative bacteria, also because of the lack of an mammalian counterpart. It catalyzes the first irreversible step of the biosynthesis of lipid A, the hydrophobic anchor of the outer membrane lipopolysaccharides (LPS) of Gram-negative bacteria. The inhibition of LpxC leads to the death of bacteria and a decrease in the amount of endotoxin released from the bacteria. [3]
In chiral pool syntheses, a series of *C*-furanosidic LpxC inhibitors has been obtained and biologically tested to explore structure-activity-relationships.
Starting from D- and L-ribose several Zn^{2+}-chelating hydroxamic acids have been synthesized. The partially desoxygenated tetrahydrofuran ring serves as a linker between the hydroxamate moiety and the lipophilic tail, that mimics the fatty acyl chain of the enzyme's natural substrate UDP-3-O-[(R)-3-hydroxymyristoyl]-N-acetylglucosamine, and leads to a comformational restricition.



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POS.64

Development and derivatization of benzhydroxamates as selective HDAC inhibitors as modulators of epigenetic targets

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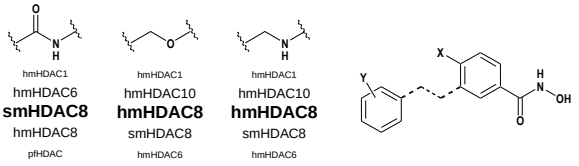
In a previous study [1], we discovered various benzhydroxamate derivatives as potent parasitic HDAC inhibitors with selectivity over the human HDAC orthologues. We could establish that 3-benzamido-benzhydroxamates showed a stronger inhibitory activity on parasitic HDACs than the 3-benzamino or 3-benzyloxy derivatives, which are showed higher potency against the tested human HDAC isoforms. In this study, we focus on the 3-benzamino derivative TH34, which was found to be a selective co-inhibitor of human HDAC8 and 10, and hence a promising candidate for the treatment of neuroblastoma. Neuroblastoma is a tumor consisting of neural crest derived undifferentiated neuroectodermal cells and is the most common solid tumor in childhood [2]. For neuroblastoma it was shown that high HDAC8 expression correlates with advanced stage disease and poor overall survival. Meanwhile, high HDAC10 expression is associated with exceptionally poor outcomes in advanced stage neuroblastoma patients [3]. The combination of HDAC8 and HDAC10 inhibition induces DNA damage-mediated cell death in human high-grade neuroblastoma cell lines [5]. The aim of this project is to develop selective HDAC8/10 inhibitors since inhibition of other HDACs causes side-effects like leukopenia, weight loss and fatigue syndrome.



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POS.65

C2-Linked Dimeric Strychnine Analogues as Bivalent Ligands Targeting Glycine Receptors: Influence of Longer Spacers

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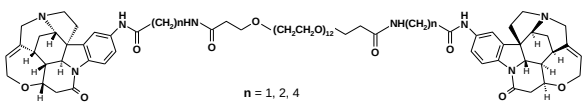
The most pronounced pharmacological action of strychnine, the major alkaloid from *Strychnos nux vomica*, is an antagonistic activity at glycine receptors (GlyRs). GlyRs are chloride channels composed of five subunits (α or β) and linked to hyperpolarisation and inhibition of neuronal firing.[1] The crystal structure of human α3 GlyR in complex with strychnine revealed five equivalent strychnine binding sites located at the interfaces of the subunits.[2] Recently, our groups have become interested in a bivalent ligand approach for targeting GlyRs. In particular, extensive series of C2-amide linked and C11-oxime ether linked monomeric[3] and dimeric strychnine analogues with spacer lengths corresponding to 6-48 atoms have been synthesized and pharmacologically evaluated at homomeric $(\alpha1)_5$ and heteromeric $(\alpha1)_2\beta_3$ GlyRs. However, none of the strychnine dimers showed higher antagonistic potency compared to the monomeric control compounds indicating that longer spacers might be needed for simultaneous binding of the two strychnine pharmacophores to two distinct binding sites. Here, we report the synthesis and pharmacological evaluation of a novel series of C2-linked dimeric strychnine analogues with spacer lengths of 51, 54, and 57 atoms. The findings are supported by docking studies at the α3 GlyR involving simultaneous binding to two adjacent strychnine binding sites.



Acknowledgments: Deutscher Akademischer Austauschdienst, Bundesministerium für Bildung und Forschung

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POS.66

A HPLC-DAD-MS method for stability tests of alkynylgold(I)(NHC) complexes in solution

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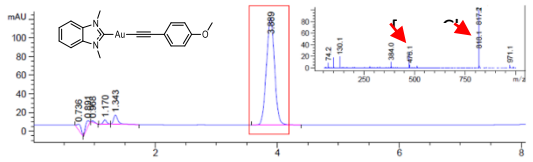
Due to their strong σ-donating character *N*-heterocyclic carbene (NHC) ligands can be coordinated well to several transition metals. This property has recently attracted major attention in inorganic medicinal chemistry, and in particular in anticancer metallodrug development. Especially gold(I) complexes have been extensively investigated and have shown a high potential regarding antiproliferative effects.[1-2] Whereas the enhanced stability has been a driving argument for the design of metal-NHC based drugs, extended studies on their solution chemistry have not been frequently performed and aspects of pharmaceutical analytics have not been considered sufficiently. We selected complexes of the type of alkynylgold(I)(NHC) for detailed stability studies by HPLC-MS.
A RP-based chromatographic method[3] was established to separate possible degradation products of alkynylgold(I)(NHC) complexes. The stability studies were performed at 37°C over 24h using dimethylformamide (DMF), dimethyl sulfoxide (DMSO), water and Dulbecco`s modified eagle medium (DMEM) solutions of each compound. The injections of the samples were fully automated and done after 4h, 8h, 12h and 24h. ESI (+) and (-) ionisation with a quadrupole analyser was used for mass spectrometry. The first results indicate that alkynylgold(I)(NHC) complexes are stable under all conditions with no significant changes in the AUCs (see figure).



Chromatogram of a selected complex in DMF after 24h

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POS.67

Synthesis and Biological Properties of Alkynyl Gold(I) N-Heterocyclic Carbene Complexes as Potential Anticancer Agents

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Several types of metal-based compounds have shown their potential as possible new anticancer agents. Among them are gold(I) based complexes, which were inspired by the disease-modifying antirheumatic drug Auranofin [1].
Thioredoxin reductase (TrxR) has been confirmed as a relevant target of gold(I) complexes. The chemical properties of gold(I) as a soft Lewis acid lead to a high affinity towards thiols or selenols, which are present in the active site of TrxR. The major role of TrxR is to reduce thioredoxin (Trx), which is required to catabolize reactive oxygen species (ROS). Importantly, TrxR is overexpressed in several tumor cell lines providing a rationale for the development of gold based anticancer agents [1].
Several types of ligands have been used to form gold(I) complexes as TrxR inhibitors. Whereas halide, thiolate and phosphane ligands had been used in the established gold lead compounds, the focus has more recently shifted towards organometallic complexes (e.g. based on *N*-heterocyclic carbenes (NHCs) or alkynes) due to their higher chemical stability [1,2,3].

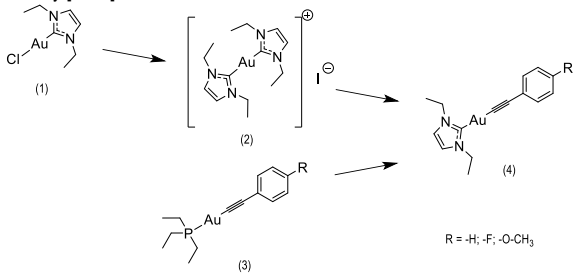

Fig. 1: Design of alkynyl gold(I) NHC complexes

NHC-gold(I) complexes (Fig. 1 (1) and (2)) demonstrated their potential as strong inhibitors of TrxR in combination with cytotoxic activity against several tumor-cell-lines, such as MCF-7 (breast adenocarcinoma) and HT-29 (colon adenocarcinoma) [2]. Alkynyl-gold(I)-phosphanes (Fig. 1 (3)) have shown a similar strong activity against the same cell lines with additional anti-angiogenic effects in zebrafish embryos [3].
Alkynyl gold(I) NHC complexes (Fig. 1 (4)) represent the combination of the above described types of gold organometallics. The complexes contain two supposedly stable "organometallic" ligands. The synthesis, characterisation and biological study of the complexes will be presented on the poster.

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POS.68

Reduction of hydroxylamine to ammonia via the molybdenum containing enzyme mARC

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Along with cytochrome b5 (CYB5B) and cytochrome b5 reductase (CYB5R3), the mitochondrial amidoxime reducing component (mARC) forms an *N*-reductive enzyme system, which is located in different tissues for example the liver, which is the most important organ for detoxification of various substances [1-3]. Two independent screening methods indicated, that hydroxylamine is an excellent substrate for the in our lab discovered mARC.
Although the role of hydroxylamine for the human organism is not completely known yet, the function of ammonia is very important due its high toxicity. Therefore, the organism developed different mechanism for detoxification of ammonia; one prominent example is the generation of urea [4]. So it is of most interest to qualify, quantify and determine the kinetic of this reduction.

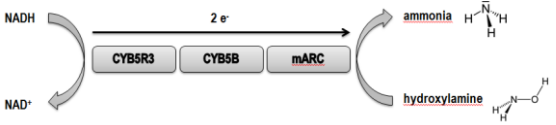

Figure 1: Conversion of hydroxylamine to ammonia by the *N*-reductive enzyme system mARC.

The biotransformation assays included different enzyme sources and hydroxylamine as substrate for the reduction to ammonia, which was quantified with an ammonia-testkit based on an enzymatic reaction located in mitochondria:

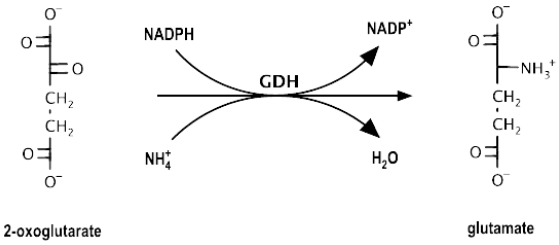

Figure 2: Enzymatic reaction of ammonia with 2-oxoglutarate via glutamate-dehydrogenase (GDH) to glutamate under consumption of NADPH [5].

We succeeded to develop a specific assay to quantify ammonia in the presence of hydroxylamine and proved that hydroxylamine is the best substrate for mARC1 and mARC2 tested so far in comparison to all previous tested substrates.

References:
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POS.69

Cyclic Voltammetry Studies on the Mitochondrial Amidoxime Reducing Component (mARC): Search for a Fast and Efficient Screening Method

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Biotransformation studies are of great importance regarding the pharmacokinetic properties of new drug candidates. In most cases the xenobiotic is oxidized or hydrolysed in phase I reactions, but reductions are observed as well, especially for amidoxime containing structures. The enzyme, responsible for the reduction of these amidoximes, was discovered 2006 and named mitochondrial amidoxime reducing component (mARC) [1]. Interestingly, while all mammals harbour two different mARC genes, the physiological role still remains unclarified.
mARC acts together with cytochrome b_5 (CYB5B) and NADH cytochrome b_5 reductase and is capable of reducing a broad range of *N*-hydroxylated structures [2]. In the last years our group used recombinant expressed mARC protein for incubation studies to discover new reducible substrates. The detection and quantification was done by HPLC analysis. But the large number of potential substrates motivated us to search for other, faster and cheaper, detection methods.
A promising tool is the cyclic voltammetry. Belonging to a redox process, reductions are accessible for electrochemical studies. A gold electrode was prepared with a modified surface and loaded with CYB5B and mARC [3]. The electrons were transferred via CYB5B and mARC on the substrate. The electrode was immersed in different solutions while the applied potential was changed and the redox response of CYB5B was monitored. Upon addition of reducible substrates by mARC, the measured current decreased and the shape of the voltammogram changed (see figure 1).
Exploiting this method, we are able to check new potential substrates of mARC very fast. In addition, different substrate concentrations can be measured and K_M values be calculated. The system works excellent for highly reduced substrates like benzamidoxime or hydroxylamine and needs to be optimised for weak substrates like fosmidomycin, where no significant current change could be detected.

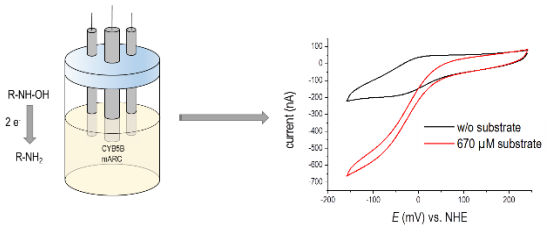

Figure 1: Design of the used electrochemical cell (modified from Elgrishi *et al.*, 2018) and representative resulting voltammogram.

References:
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POS.70

New chemical entities targeting MKK4 to increase regeneration of hepatocytes

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2School of Pharmacy, University of Eastern Finland, Kuopio, Finland
3Divison of Translational Gastrointestinal Oncology, Department of Internal Medicine I, University of Tuebingen, Tuebingen, Germany

Using a pool of small hairpin RNAs (shRNAs) MKK4 (mitogen-activated protein kinase kinase 4) was identified to be a major regulator of liver regeneration. Silencing of MKK4 increased the robustness and regenerative capacity of hepatocytes during acute and chronic liver failure in a mouse model experiment. Mechanistically this leads to a higher phosphorylation of MKK7 and JNK1 and an enhanced activation of the transcription factors ATF2 and ELK1, causing a faster replication and less apoptosis and fibrosis of hepatocytes. [1,2]

Our approach developing small molecules as inhibitors for MMK4 is based on a virtual screening. Pdb-3ALO structure was selected as a target for molecular docking. The compound libraries used were general hit-identification libraries and we specifically decided not to use any kinase-targeting libraries. Since no X-ray structure of phosphorylated MKK4 exists the models are only valid for type-2 inhibitors but this do not rule out also type-1 inhibitors. The used docking methods were Glide and SurflexDock and in both cases the docking site was based on the ATP-binding region of MKK4. As the Mg2+ ion was included in the X-ray structure (3ALO) it was included also in the docking experiment. In both methods docking was carried out using incremental approach, so at first screening with fast approximated scoring/pose scanning and at later stated with more robust/time consuming settings. The combined docking resulted 180 compounds for purchase and in vitro testing. In our in-house in vitro assay approx. 55 % of the compounds showed detectable MKK4 inhibition. To validate the hits we also used DiscoverX® in vitro assay for a selected inhibitors resulting POC (percent of control) of 5 @ 10 µM at a screening at and a good selectivity profile, but lacking chemical stability. The chemically stable derivative was determined with a POC of 23 @ 10 µM. Further derivatisation and optimization resulted in a compound with a POC of 25 @ 1 µM.

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POS.71

Metabolic Stability Determination *in vitro* of Novel Type I ½ p38α MAP Kinase Inhibitors

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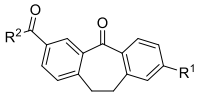
Besides selectivity, potency and target residence time, type I ½ inhibitors should reveal a suitable metabolic profile. In order to examine if and to what extent the variety of residues influences metabolic stability, the in vitro biotransformation of selected dibenzosuberone and dibenzooxepinone inhibitors was investigated in male rat liver microsomes (RLM). Furthermore, the hypothesis of the potentially blocked metabolic position of the dibenzosuberone scaffold **A** with the introduction of an oxygen leading to the dibenzooxepinone scaffold **B** was investigated (Fig.1). Therefore, the compounds profiled (Tab. 1) reflected the structural diversity and revealed remarkable results in the RLM assay. All tested substances appear to undergo metabolic degradation and reveal metabolite formation. The introduction of the cyclopropyl residue as an anilide (**2**) led to an increased metabolic stability compared to the reverse amide (**1**). To our surprise, the insertion of a heteroatom to the scaffold A led to decreased metabolic stability and increased metabolite formation when compared with its dibenzosuberone equivalent (**3** vs **4**).



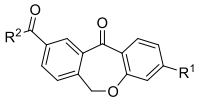
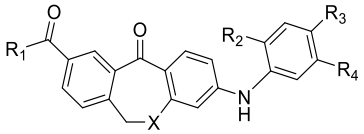


Figure 2: Scaffold **A**: dibenzooxepinone Scaffold **B**: dibenzosuberone



	R_1	R_2	R_3	R_4	X	Metabolic stability [%] ± SD
1		H	F		CH_2	61.6 ± 2.2
2		H	F		CH_2	79.5 ± 6.8
3		H	F		CH_2	40.6 ± 5.9
4		H	F		O	14.8 ± 0.9

Table 1: Metabolic Stability of Selected Dibenzosuberone Inhibitors **1**, **2**, **3** and Dibenzooxepinone Inhibitor **4** after 180 min Incubation with RLM

Walter NM, Wentsch HK, Bührmann M, Bauer SM, Döring E, Mayer-Wrangowski S, Sievers-Engler A, Willemsen-Seegers N, Zaman G, Buijsman R, Lämmerhofer M, Rauh D, Laufer SA. Design, Synthesis, and Biological Evaluation of Novel Type I(1)/(2)p38α MAP Kinase Inhibitors with Excellent Selectivity, High Potency, and Prolonged Target Residence Time by Interfering with the R-Spine. J Med Chem. 2017 Oct 12;60(19):8027-8054. doi: 10.1021/acs.jmedchem.7b00745.

POS.72

Synthesis and biological evaluation of imidazole and pyrazole derivatives of Deferasirox targeting JmjD2A (KDM4A) demethylase

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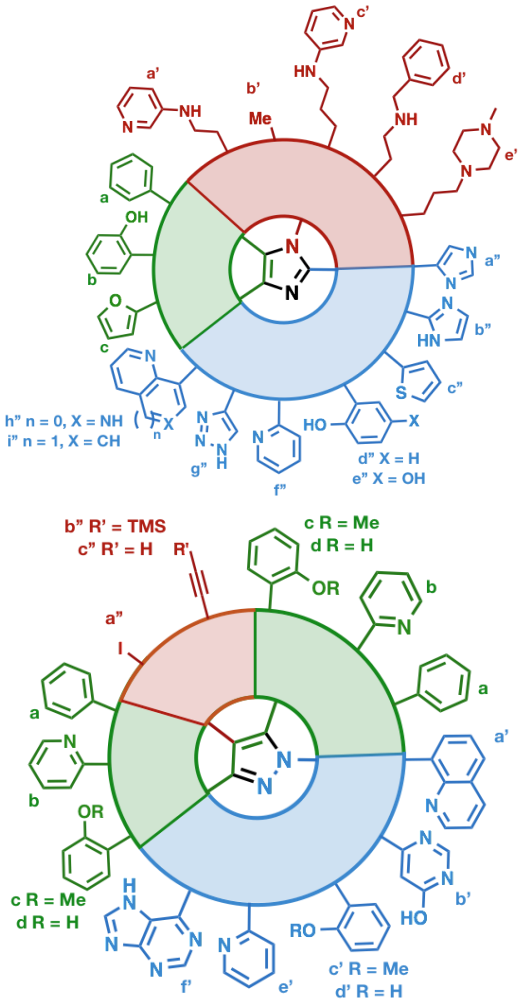
KDM4A, a lysine specific demethylase belonging to the family of the JumonjiC domain-containing demethylases (JmjC), specifically recognize the trimethylated lysine residues H3K9 and H3K36 and demethylate them thanks to its Fe(II) center and α-ketoglutarate (αKG) and molecular oxygen O2 used as co-substrates.[1] Poorly expressed in normal cells, Its overexpression has been observed in different cancer tissues (e.g. lung, breast, colon), making it an highly promising epigenetic target.[2]
Since KDM4A depends on an iron in its active site for its enzymatic activity, our group tested clinically used iron-chelator drugs in order to identify new motives enable to target this demethylase. More particularly, deferoxamine mesylate (approved in 1968), deferasirox (approved in 2005), and deferiprone (approved in 1994 for Europe & Asia and in 2011 for the USA) were tested and appeared to be highly potent in vitro, with IC50 in the low micromolar range. However, our results suggested that deferoxamine and deferiprone, in contrast to deferasirox, seem to inhibit the enzyme solely by sequestration of the iron ions in solution and not by

competing with αKG. Because of its high affinity for KDM4A, deferasirox was selected and analogues were synthesized, but, unfortunately, without increase in the potency. Later on, thiazole derivatives of deferasirox, exhibiting activity in low micromolar range as well, were also synthesized.[3]



In this context, a library of imidazole derivatives of deferasirox was prepared using a multicomponent approach. Contrary to the former families synthesized, imidazoles have the advantage of bearing a side chain, allowing them to generate more interaction within the active site, and bringing high potential for the future of this family. On the same model, pyrazoles, isomers of imidazoles, were also prepared. Iodination and subsequent substitution by side chains can be performed on these aromatic moieties to increase their interaction with the target demethylase. Herein, the synthesis of such derivatives and the first biological results will be presented.

We thank for funding by the DFG (CRC992 Medical Epigenetics) – Project A04

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POS.73

Autodisplay of human cytochrome P450 enzymes 3A4, 1A2, 2C9, 2C19 and 2D6 and cytochrome P450 reductase for drug metabolite studies

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Cytochrome P450 monooxygenases (CYPs) are responsible for the biotransformation of most drugs and xenobiotics in human body[1]. As part of the phase-I-metabolism they catalyze a broad diversity of oxidation reactions in an extensive spectrum of substrates. The utilization of CYPs as biocatalysts is limited due to their low stability and their requirement of a membrane surrounding to fold into an active form[2]. Autodisplay of CYPs on the surface of *E. coli* has been shown an appropriate tool to overcome these limitations[3,4]. In order to establish an *in vitro* system to study drug metabolism, the five most important CYPs, CYP 3A4, CYP 1A2, CYP 2C9, CYP 2C19 and CYP 2D6 were displayed on the surface of *E. coli*. The catalytic activity of CYP 3A4 was shown by testosterone as a substrate using a HPLC assay with external addition of the electron supplying enzyme cytochrome b5[5]. A co-expression of CYP 1A2 and cytochrome P450 reductase (CPR) was established with both enzymes being displayed on the surface of *E. coli*. Surface display was confirmed by a protease accessibility test and by flow cytometry. Activity of surface displayed CPR was additionally analyzed with cytochrome c as substrate. Surface displayed CYP 1A2 with co-expressed CPR showed conversion of phenacetin to paracetamol and of 7-ethoxyresorufin to the fluorescent product resorufin[6]. CYP 2C9, CYP 2C19 and CYP 2D6 were co-expressed with CPR on the surface of *E. coli* as well. Surface display could be confirmed via SDS-PAGE and Western Blot. Combining cells with these five CYP enzymes in an active form on the bacterial cell surface is supposed to provide a suitable approach for the *in vitro* simulation of drug metabolism.

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POS.74

C-pyranosides as inhibitors of the bacterial deacetylase LpxC

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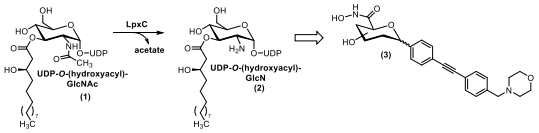
Despite the numerous antibiotics, which have been developed since the discovery of the first antibacterial compounds in the first half of the last century, today the need for novel classes of antibiotics is a topic with immense importance. The reason for this is caused by an increasing number of bacterial pathogens being resistant against the major classes of commercially available antibacterials.[1] Besides causing high economic cost, infections with resistant pathogens claim a large number of human lives.[2]
In order to treat infections caused by multiresistant Gram-negative bacteria, the inhibition of the bacterial deacetylase LpxC is a promising but so far clinically unexploited strategy. LpxC is an essential enzyme in the biosynthesis of Lipid A, which is the hydrophobic anchor of lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria. LpxC is highly a conserved, cytoplasmic enzyme, which is required for growth and viability of Gram-negativ bacteria.[3,4] The Zn^{2+}-dependent enzyme catalyzes the commited step of Lipid A biosynthesis, the irreversible deacetylation of UDP-3-*O*-[(*R*)-3-hydroxyacyl]-*N*-acetylglucosamin **(1)**.



LpxC inhibitors generally exhibit a Zn^{2+}-chelating hydroxamate moiety and a lipophilic side chain mimicking the fatty acyl chain of the natural substrate **1**.[4] Inspired by the glycosidic nature of substrate **1**, we designed a series of stereoisomeric *C*-pyranosides **(3)**, whose synthesis and biological evaluation will be presented herein.

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POS.75

Synthesis and Evaluation of Novel Covalent-Irreversible JAK3-Inhibitors with high Isoform-Selectivity Based on a Conformational Rigid Tricyclic Hinge-Binding-Motive

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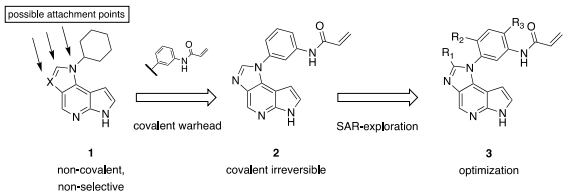
The Janus kinase (JAK) family is highly involved in cytokine signalling and plays an important role in crucial physiological processes like cell proliferation, differentiation, migration and apoptosis. While all JAKs are cytosolic nonreceptor tyrosine kinases, three of the four family members (JAK1, JAK2 and TYK2) are ubiquitously expressed. However, JAK3 fulfils its important tasks only in cells of the immune system like B-, T- and NK cells.[1] Recently approved JAK inhibitors like Tofacitinib and Baricitinib are therefore used in treatment of inflammatory diseases due to their immunosuppressant effects.[2, 3] However, the insufficient selectivity of these drugs within the JAK family results in diverse side effects. This lead to the idea of highly selective JAK3 inhibition as strategy to overcome the detriment of these therapeutic agents.



Within our medicinal chemistry project for the development of novel JAK3-selective inhibitors, we identified compound **1** (X = N) as moderately potent (IC_{50} < 100 nM), but unselective JAK3 inhibitor.[4] One possibility to achieve a more beneficial isoform selectivity is the targeting of a non-conserved cysteine (C909) nearby the ATP binding pocket, which can only be found in the JAK3 instead of a serine compared to the other family members.[3] Our approach was to develop irreversible inhibitors by introduction of a covalent phenylacrylamide warhead at different attachment points on the low molecular weight scaffold of **1** in order to achieve high selectivity within the JAK-family. These efforts culminated in the identification of compound **2**, a highly potent and selective JAK3 inhibitor (IC_{50} < 10 nM), which most probably irreversibly addresses C909. Further derivatisation of this lead allowed to explore the SARs of this inhibitor class and even resulted in additional selectivity within the JAK family.

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POS.76

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

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

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POS.77

3,5-disubstituted 7-Azaindoles as Novel Low Molecular Weight Scaffold for the Development of Irreversible JAK3 Inhibitors with High Isoform Selectivity

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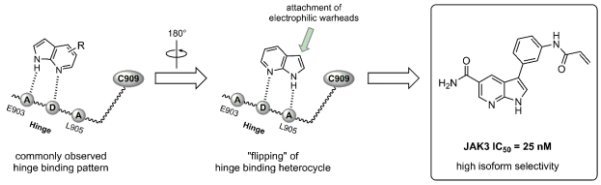
The physiologically important processes of proliferation, differentiation and maturation of haematologic cells are mainly driven by cytokines like interleukins (ILs), interferons (IFNs), and growth factors. The signal pathways initiated by these extracellular stimuli are often mediated by the Janus kinases (JAKs). This kinase family, consisting of four closely related isoforms (JAK1, JAK2, JAK3 and TYK2), is a key player in the regulation and homeostasis of the immune system.[1] Since JAK3 is the

only family member, which is exclusively operating in immunocompetent cells, it earned a special attention in the last years, as a promising target for the development of a new class of immunosuppressants.[2]
Due to the high structural similarity of the four JAKs, the development of isoform specific inhibitors is a challenging task. However, another unique feature of JAK3 is the presence of a non-conserved cysteine residue (C909) nearby the ATP binding site, which is replaced by a serine in the three other family members. A high JAK3 selectivity can be therefore achieved by the targeting of C909 with electrophilic inhibitors, a strategy that has already been successfully applied by our group as well as others.[3-5]



In a new approach to develop irreversible JAK3 inhibitors, we postulated that an alteration of the commonly observed hinge-binding pattern of purine isosteres would favor the 3-position of the five-membered pyrrol ring as possible attachment point for electrophilic warheads to target C909. Following this strategy, we were able to synthesize a small set of potent JAK3 inhibitors with a high isoform selectivity. The expected binding mode was also successfully confirmed by XRay crystallography and the low molecular weight of this scaffold provides the opportunity to further develop physicochemical properties in ongoing optimization cycles.

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POS.78

Synthesis and antibacterial properties of glyceric acid derivatives as LpxC inhibitors

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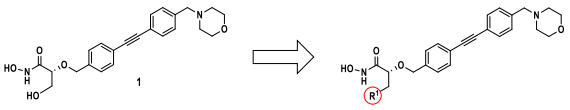
The rise of multidrug-resistant Gram-negative bacteria and the lack of new effective classes of antibiotics in the clinic is one of the most important threats to global public health. Therefore, to avoid a post-antibiotic era in which common infections could once again lead to death, new antibacterial agents addressing innovative bacterial targets are urgently needed [1].
A promising strategy to combat these bacteria represents the inhibition of the Zn^{2+}-dependent deacetylase LpxC, an essential enzyme of the biosynthetic pathway of lipid A, the membrane anchor of lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria. As the inhibition of the biosynthesis of lipid A is lethal to Gram-negative bacteria, inhibitors of LpxC represent a promising class of novel antibiotics [2].
Glyceric acid derivative **1** is a potent inhibitor of LpxC and served as lead structure for the development of novel inhibitors. In these new compounds, the hydroxy group in Cβ position of the hydroxamate moiety was replaced by functional groups, which on the one hand can participate in hydrogen bond formation and on the other allow the attachment of residues addressing the UDP-binding pocket of LpxC. The envisaged LpxC inhibitors were synthesized in chiral pool syntheses starting from D-mannitol and tested for antibacterial activity.



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POS.79

Essential Replication Protein A targeting by PPI Inhibitors for cancer treatment

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The eukaryotic single-stranded (ss) DNA-binding protein, replication protein A (RPA), is a central player in DNA metabolism [1]. During the cell cycle, it is involved in replication, recombination and repairing processes to ensure orderly completion of genome duplication. RPA is a heterotrimeric complex consisting of three subunits, namely RPA1, RPA2 and RPA3, which, once assembled, act as the most important ssDNA binding protein. The RPA-complex controls the global response of new origin firing during the replication process via the ATR-CHK1 pathway, therefore being is responsible for genomic stability [2]. Toledo et al. further underlined that a progressive RPA depletion after ATR inhibition leads to massive DNA breakage due to exposure of unprotected ssDNA within the replication forks, ultimately leading to cellular stalling.
When targeting an essential cellular process for cancer therapy, a therapeutic window might occur either because inhibition of an essential process induces a stress response specifically or predominantly in cancer cells but not in normal cells. This happens because the respective cellular process is upregulated in cancer cells resulting in a higher dependency on the process in cancer- vs. normal cells. Since many cancers harbor high levels of replicative stress the inhibition of the RPA-complex should result in the exposure of unprotected ssDNA with subsequent accumulation of double-strand DNA breaks and cell death.
Aiming for a clinically available treatment, pharmacological RPA3-reduction was sought by small molecules. A first lead compound was identified by *in silico* screening approaches and validated by EMSA assays, where it interfered with DNA interaction with moderate activity.
Despite the promising potency, the compound poor drug-likeness and physicochemical properties required further chemical optimization. Accordingly, the lead compound was shortened to its core essential elements using molecular modelling techniques and, by the means of a fast learning iterative cycle, we could optimize this lead compound to our first generation. This new compound series is suggested to act as a protein-protein interaction inhibitor. Phenotypically the compound could mimic the effect of shRNA application on a cellular level, reducing the RPA3 interaction with genomic DNA.
Additional improvement aiming to increase the potency against pancreatic cancer cell lines lead to tool-compounds with IC_{50} values on the two-digit nanomolar range and impressive preliminary pharmacokinetic properties in mice. As a perspective, we now intend to further optimize ADME-properties, which ultimately can lead to a clinical candidate for the treatment of aggressive and fast proliferating cancers.

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POS.80

PST.01

Peptidomimetic β-lactams as electrophilic warheads in flaviviral protease inhibitors

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Introduction

The ability of β-lactam ring to form an irreversible covalent bond with a catalytic serine of transpeptidases and β-lactamases is the basis of the β-lactam antibiotic activity.[1] On the other hand, β-lactams serve as peptidomimetic moieties, which are, among other things, used in the development of pharmaceutically active peptides.[2] The idea behind the project was to combine the two properties of β-lactams in order to create peptidomimetic β-lactam warheads, which would then be used as inhibitors of flaviviral protease. Flaviviral protease is a trypsin-like protease with a catalytic triad containing histidine, aspartate and a catalytic serine.[3] New β-lactam peptidomimetic moieties were introduced instead of C-terminal amino acid of previously developed peptide inhibitors (1) of dengue virus (DENV) NS3 protease.[4] (Figure 1).

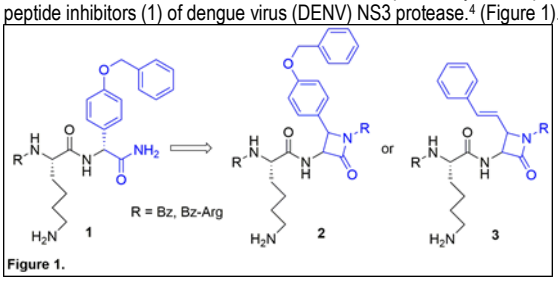

Figure 1.

Results and discussion

β-Lactams were obtained applying the enolate-imine cyclocondensation reaction.[5] This method facilitates the synthesis of all four β-lactam isomers in enantioselective or diastereoselective fashion. The absolute configurations of β-lactam isomers were assigned using electronic circular dichroism (ECD) spectroscopy and time-dependent density functional theory (TDDFT) calculations.[6] Single isomers of β-lactams were coupled to C-terminus of either N-benzoyl-capped lysine or N-Bz-Arg-Lys dipeptide. In vitro activity of new peptide-β-lactam derivatives was tested against DENV protease, West Nile virus (WNV) protease, thrombin and trypsin. The highest activity against DENV and WNV NS3 protease showed derivatives containing β-lactams with 4-benzyloxyphenyl (2) and cinnamyl moiety (3) at C-4 position. The activity of tested derivatives was very dependent on the configuration of β-lactam. In both cases, enantiomer cis-(3S,4R)- showed the highest activity. The activity in WNV assay had the same trend. Tested compounds showed no activity against off-target thrombin and trypsin. Although compounds cis-(3R,4S)-2 and cis-(3R,4S)-3 had very similar IC_{50} value (i.e. 7.0 and 8.9 μM, respectively), they exhibited very different mechanism of action. Inhibition mechanisms for both compounds were determined using Dixon and Cornish-Bowden plots[7] and a biochemical assay for time-dependent inhibition in order to characterize the reversibility of the binding.[8] Compound 2 showed non-competitive/mixed inhibition mechanism and irreversible binding mode to DENV protease, whereas 3 exhibited competitive mechanism and reversible binding.

Conclusion

β-Lactam inhibitors of flaviviral protease have been developed by combining the peptidomimetic and electrophilic properties of β-lactams. New compounds exhibited inhibition in low micromolar range with the inhibition rate strongly depending on the configuration of β-lactam ring. The mechanism of action varied regarding the substituents on the β-lactam ring. The reasons behind the differences in the mechanism of action remain to be elucidated. Moreover, the binding of compound 2 has to be further characterised with regards to covalent inhibition.

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POS.81

Exploring hit-identification strategies for energy-coupling factor transporters, a novel target for the development of antibiotics

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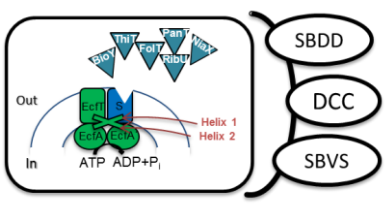
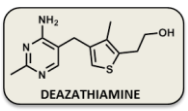
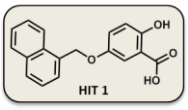
The emergence of drug resistance against important pathogens poses an ever-growing health threat. The pipeline of novel drug candidates should be filled with molecules featuring an unprecedented mode of action and an unprecedented chemical structure. We address both challenges by using multiple hit-identification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

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

A structure-based virtual screening (SBVS) provided us with the first allosteric inhibitors of the transporter for folate able to both reduce folate concentration in the cytosol and to reduce the bacterium growth. Additionally, the excellent drug-like properties of this chemical class of compounds triggered a medicinal chemistry campaign that turned out with the first inhibitors against the ECF transporters active against a plethora of pathogenic Gram-positive organism (Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium).[5]







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POS.82

Indirect MYC targeting via conformation changing Aurora Kinase A inhibitors

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Hepatocellular Carcinoma (HCC) – the second most frequent cause of cancer-related death - has a great need for better therapeutic strategies since the treatment options for advanced HCC are limited. Furthermore, the rapid emergence of resistance against existing treatments demonstrates the necessity for novel therapeutic approaches. [1,2]
In previous publications and preliminary work, it was shown, that transient systemic MYC inhibition serves as a suitable treatment option for HCC. [3] However, due to its lack of binding cavities MYC cannot be targeted directly by small molecules. By using a direct *in vivo* shRNA screen, recent findings showed that liver cancer cells bearing mutations in the gene encoding the tumor suppressor protein p53 (Trp53 in mice and TP53 in humans) and that are driven by the oncoprotein NRAS become addicted to MYC stabilization via a mechanism mediated by aurora kinase A (AURKA). [4] Therefore, an indirect MYC targeting becomes possible.
Our work focusses on the design and synthesis of small molecules that prevent MYC/AURKA complex formation by inducing a conformational shift in the kinase. *In silico* predictions propose that the crucial interaction for complex formation is located in close proximity to the kinases hydrophobic spine. Hence we make use of the already in our group successfully applied concept of type I ½ inhibitors. Guided by *in silico* approaches and further mechanistic studies we aim to develop tool compounds providing structural features that are able to prevent MYC/AURKA complex formation and ultimately degrade MYC.

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POS.83

Facilitating lipidic cubic phase crystallization experiments with new opioid receptor agonists

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The opioid receptor family, which is endogenously activated by a group of peptides (enkephalins, endorphins, dynorphins), consists of three different G protein-coupled receptor (GPCR) subtypes: μ-, κ- and δ-opioid receptor (MOP, KOP and DOP, respectively). While approved drugs which act by unselective opioid receptor activation are unrivalled in their analgesic potential, the treatment is associated with the risk of serious adverse effects (addiction, tolerance, respiratory depression). As a consequence, the USA is currently challenged with an opioid crisis, resulting in opioid overdose as the dominating cause of accidental deaths. [1] Both G protein-biased ligands [2] and allosteric modulators [3] for the MOP have become a promising directive to overcome undesired opioid effects whereas KOP and DOP selective agonists have shown new potential for the treatment of mood disorders, chronic pain and opioid withdrawal. Recent advances in GPCR structural biology have been increasing the knowledge of opioid receptor binding pockets and activation mechanisms [4], aiding the development of new opioid receptor ligands based on different chemical scaffolds. However, only a small number of agonist-bound opioid receptor crystal structures have been solved thus far, all of them in complex with morphinan-based agonists and without coverage for the δ-opioid receptor. [5] This study is focused on evaluating stabilizing effects of agonists with different chemical scaffolds on opioid receptor mutants in pre-crystallization assays to discover promising new ligands for lipidic cubic phase crystallization experiments.

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POS.84

Hyaluronidase-1 inhibitors provided by nature – marshmallow root extract effecting Hyal1 on protein and gene level

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The endoglycosidase hydrolase Hyaluronidase 1 (Hyal1) is one of five functional hyaluronidases in human body. Degradation of high molecular weight hyaluronan (HA) is mainly catalysed by Hyal1. The resulting low molecular weight fragments have inflammatory and angiogenic effects.[1] In cancer progression, e.g prostate or bladder, the role of Hyal1 has been discussed for a long time. In several cancer cells, the expression level of Hyal1 was elevated in comparison to not malignant cells, resulting in higher Hyal1 activity and tumor progression.[2,3]
Hyal 1 is an interesting target for pharmaceutical purposes, but no potent inhibitors have been found so far. The enzyme source seems to be the bottleneck in investigation of potent inhibitors. Production of active Hyal1 is one of the most challenging tasks. Eukaryotic extraction and purification is very time consuming and expensive. Recombinant expression in bacteria leads to inactive Hyal1 forming inclusion bodies.
Using Autodisplay, we are able to express active human Hyal1 on the surface of Escherichia coli.[4] By application of a whole cell inhibitor screening assay, we determined marshmallow root (Althaea officinalis) extract as Hyal1 inhibitor. The IC_{50} was determined to be 7.7 mg/mL. An effect of marshmallow root extract on hyal1 gene expression in eukaryotic HaCaT keratinocytes was shown in further studies. Treating the cells with 125 µg/mL extract led to a decrease in hyal1 expression by a factor of two.[5]

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POS.85

From 2-Alkylsulfanylimidazoles to 2-Alkylimidazoles: An Approach towards Metabolically More Stable p38α Mitogen-activated Protein Kinase Inhibitors.

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The p38α mitogen-activated protein kinase (MAPK) is a ubiquitously expressed serine/threonine kinase, which is crucially involved in a variety of cellular processes such as proliferation, cell survival, and differentiation. As a result, p38α MAPK gathered a lot of attention as a potential drug target with a suggested role in the pathogenesis of diseases such as multiple sclerosis and Alzheimer's disease [1,2]. The tri-substituted imidazole-based inhibitors ML3403 and LN950 display IC_{50} values in the low nM range but have unfavourable properties regarding their metabolic stability [3].
To decrease metabolic degradation, sulfanyl groups present in both ML3403 and LN950 were replaced by methylene groups leading to compound 1 (derivative of ML3403) and compound 2 (derivative of LN950). Subsequently, these derivatives were evaluated regarding their metabolic stability in human liver microsomes, their IC_{50} values in an ELISA based assay [4], and their binding affinity in a fluorescence polarization (FP)-based binding assay [5]. Both derivatives showed a vastly improved metabolic stability compared to their sulfanyl analogs.
Compound 1 displayed 20 % degradation after 4 hours incubation time (ML3403: >80 %) and compound 2 displayed merely 10 % degradation after 4 hours (LN950: > 70%). The IC_{50} value of compound 1 slightly improved compared to ML3403 from 40 nM to 25 nM. Compound 2 remained unaffected towards inhibition of p38α MAPK compared to LN950, both derivatives displayed significantly decreased Ki-values in the FP assay. TNF-α release was in both cases slightly decreased [6].

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POS.86

The *MARC2* KO mouse as a powerful tool to study *N*-reductive metabolism

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The mitochondrial amidoxime reducing component mARC is a molybdenum-containing enzyme which, in concert with the electron transport proteins cytochrome b_5 and NADH cytochrome b_5 reductase, is involved in drug metabolism [1,2]. All annotated mammalian genomes harbor two mARC genes (*MARC1, MARC2*), which share a high degree of sequence similarity. Both molybdenum enzymes act as a reductase towards a variety of *N*-oxygenated compounds with overlapping substrate specificity [3,4].
In the present study knockout mice which lack the functional *MARC2* gene were obtained from the European Mouse Mutant Archive. These mice were viable and fertile, and did not exhibit any obvious physiological deficiencies. Striking difference was a significant lower body weight of KO mice compared to control and an excessive hair loss.
To further investigate the function of mARC in lipid metabolism these mice were characterized regarding their total body mass under normal diet (ND) and high fat diet (HFD). Additionally, mRNA-levels as well as enzyme levels of proteins involved in lipid metabolism were examined more closely. It could be seen that all KO mice undergo a significant decrease in body weight compared to WT mice. These observations are supported by changes in mRNA and protein expression levels of proteins which are substantially involved in lipid metabolism.
To study the biotransformation reactions in more detail, tissue homogenates were prepared and incubated with several *N*-oxygenated substrates. In contrast to WT samples, liver homogenates originate from KO mice undergo a massive decrease up to total loss in reductase activity. As mARC1 and mARC2 showed overlapping substrate specificities our results indicate that mARC2 seems to be mainly responsible for their murine *N*-reduction. Residual *N*-reductive activity could be explained by unaffected mARC1 expression in KO mice.
In particular, the deletion of the *MARC2* gene can help to understand more about mARC2 phenotypes and roles in some physiological and pathological conditions as well as differences to mARC1.

We thank Petra Köster[1] and Sven Wichmann[1] for technical assistance.

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POS.87

Design and development of novel LpxC inhibitors by use of a interligand NOEs based fragment screening.

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It is a known fact that bacteria develop resistance to antibiotics at incredible speed. The number of effective antimicrobial agents available to treat multidrug resistant Gram-negative infections is steadily becoming more limited. There is therefore a great need for novel antimicrobial agents addressing so far unexploited bacterial targets [1].

Functioning as an extraordinarily effective permeability barrier, the lipopolysaccharides (LPS), which are the main component of the outer monolayer of the outer membrane of Gram-negative bacteria, play a fundamental role for the viability of Gram-negative bacteria. Inhibiting the biosynthetic pathway of lipid A, which anchors the LPS in the membrane, represents therefore a favorable strategy. Lacking homology to mammalian enzymes and catalyzing the first committed step of lipid A biosynthesis, the deacetylase LpxC represents a promising target for the development of novel antibiotics [2].

In this work novel LpxC inhibitors were designed and optimized using a fragment-based drug design approach, NMR experiments were used to screen a library of fragments in the presence of a known inhibitor and the LpxC enzyme. Interligand NOE signals were used for the identification of fragments binding to the enzyme in close proximity to the known inhibitor. This fragments were then incorporated in the design of novel LpxC inhibitors [3].

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POS.88

Submission withdrawn.

POS.89

Submission withdrawn.

POS.90

PET-radiotracer ^{68}Ga-DMALTO selectively identifies bacterial infection from aseptic inflammation

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The differentiation between bacterial infections and aseptic inflammations is one of the major challenges in post-operative patient care in clinical routine. Current diagnostic tools only poorly address this issue. A promising approach is to target bacteria specifically by their unique energy substrate maltohexaose, which is taken through maltodextrin-transporters, expressed by bacterial and not mammalian cells. Following this concept, we developed a radiolabelled maltohexaose and evaluated it *in vitro* and *in vivo* using PET imaging.

An azido-functionalizes maltohexaose was linked to the DOTA-chelator via Cu(I)-mediated click-chemistry, and labelled with ^{68}Ga. The new ^{68}Ga-DOTA-maltohexaose (DMALTO) was evaluated *in vitro* in cultured *E. coli*, *B. subtilis* and human macrophages (negative control). Three mouse models were established based on C57BL/6 mice: healthy mice, hind limb infection with wildtype *E. coli* (10^7 CFUs) and hind limb sterile inflammation induced by lipopolysaccharides (LPS, 1 mg/kg). 24h after an intervention, DMALTO (11.0±1.6 MBq) was injected via a tail vein, and dynamic PET was acquired over 60 min (Siemens Inveon µPET/CT). In another group of hind limb infected and inflamed mice ^{18}F-FDG scans (8.3±0.8 MBq) were acquired as reference and for comparison. *In vivo* metabolites of DMALTO were analysed in blood and urine by radio-TLC and radio-HPLC. Morphology and cell content of the calf muscle were determined by immunohistology.

DMALTO was radiolabelled with ^{68}Ga in excellent radiochemical yields of ≥ 95%. Cultured wildtype *E. coli* and wildtype *B. subtilis* show specific uptake of DMALTO, whereas negligible uptake was found in human macro-phages. PET studies in healthy mice demonstrated favourable pharmacokinetics, including rapid renal clearance, low background signal and the absence of radiometabolites in blood or urine (60 min p.i.). In mice with a hind limb infection of wildtype *E. coli* and *B. subtilis* (both confirmed by histology), DMALTO clearly distinguished significant (P<0.001) between the infected and non-infected injection site (ratio of up to 5.8).

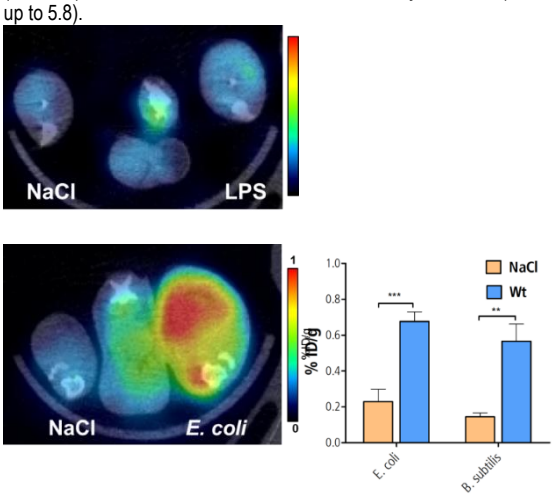

Figure 1: PET images using ^{68}Ga-DMALTO in different mouse models of bacterial infection and sterile inflammation: (left) transversal plane of both hind limbs of a mouse with a sterile inflammation (LPS) and *sham* (saline injection, neg. control); (middle) transversal plane of both hind limbs of a mouse with a bacterial infection (*E. coli*) and *sham* (saline injection, neg. control); (right) quantitative analysis of the PET imaging data for *E. coli* and *B. subtilis* infection.

By contrast, the clinically used standard ^{18}F-FDG (non-specific) could identify the infection, but had a much lower infected to non-infected muscle ratio than DMALTO. Moreover, the specificity of the DMALTO signal was tested in the sterile inflammation model (confirmed by histology). While ^{18}F-FDG uptake remained prominent (LPS: 1.48 %ID/cc, saline: 0.67 %ID/cc, P<0.01), reflecting the local inflammation and no specificity, DMALTO uptake was similar to the non-inflamed contralateral muscle (LPS: 0.38 %ID/cc, saline: 0.25 %ID/cc, P>0,05).

A new infection diagnostic imaging biomarker was developed, capable of distinguishing between wildtype *E. coli* and *B. subtilis* infection and sterile inflammation in vivo. Moreover, DMALTO identifies gram(-) (*E. coli*) as well as gram(+) (*B. subtilis*) bacteria. Further exploration of the potential of DMALTO as specific bacterial infection marker in other animal models and the translation into humans are warranted.

3.3 Antiinfectives

POS.91

Influence of the second-shell amino acid 121 on the Zn^{2+} binding affinity in the active center and catalytic activity of metallo-β-lactamases

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Metallo-ß-lactamases (MBL's) are a major problem in the therapy of bacterial infections, due to their capability of inactivating a wide variety of ß-lactam antibiotics. The increased spread of pathogenic bacteria producing these enzymes as well as the lack of clinically approved inhibitors further increase the problem.[1,2] Therefore it is important to investigate the underlying mechanisms, with the aim to find broad acting inhibitors, which can be used to regain the effects of antibiotics against these pathogenic bacteria.
MBL's are divided in three subclasses (B1,B2,B3) according to their sequence identity.[3] Members of the subclass B1 can bind one, or more likely two, Zn^{2+} ions in their active center which are coordinated by the amino acids His116-His118-His196 and Asp120-Cys221-His263.[4,5] As demonstrated in previous publications, these binding motives are strongly conserved in the subclass. Nevertheless, the variety of the hydrolyzed ß-lactam antibiotics as well as the hydrolysis rate are quite diverse for the different members of the subclass and are influenced by a broad range of factors including diverse loop regions and variations in the second-shell of the proteins.[6,7]
An interesting member of the B1 subclass is the NDM-family, containing 17 enzymes.[8] These enzymes are known to hydrolyze a larger number of ß-lactam antibiotics with a higher rate than other B1 enzymes, but are often not as stable, nor do they bind the Zn^{2+} ions as strongly as other MBL's.[9,10] One approach to understand this effects is by an analysis of the second-shell.
The second-shell consists of amino acids that have an influence on the binding of Zn^{2+} ions without directly interacting with them.[11] One amino acid of the second-shell has been shown to be quite variable between the different B1 enzymes, but is a conserved lysine in the NDM-family. This is amino acid 121. Several crystal structures of MBL's show that amino acid 121 influences the Zn^{2+} coordinating amino acids.[6] These aspects make the position 121 a very interesting target to evaluate its effect on the Zinc binding and the ß-lactam hydrolysis by mutation studies. We propose that the better hydrolyzing capacity as well as the impaired stability shown by NDM are caused by the lysine 121.
We performed a cross mutation of the amino acid 121 with four prominent members of the subclass B1 (NDM-1, VIM-2, IMP-1 and SPM-1) and evaluated the protein stability with several Zinc concentrations via differential scanning fluorimetry (DSF) experiments as well as the binding affinity of the two Zn^{2+} ions via isothermal titration calorimetry (ITC).
We were able to show that mutating amino acid 121 has a significant effect on the binding affinity towards the Zn^{2+} as well as an effect on the protein stability in correlation to the Zinc concentration. The influence of the mutations towards the kinetics of the ß-lactam antibiotics are ongoing research.

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POS.92

Investigation on Transferability of Antibiotic Resistance Plasmids in a Multiple Drug Resistant *E.coli* ST131 Isolate

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Introduction:
The prevalence and spread of multidrug resistant (MDR) bacteria has increased rapidly over the last years.
Genes mediating antibiotic drug resistance can be located either in the genome of bacteria or on plasmids [1] and therefore might be transferable to other bacteria of the same or even between species.
One of the globally spreading lineages of high risk clones with multiple antibiotic drug resistance is *E. coli* of Sequence Type (ST) 131 [2]. Thus, this investigation focused on the localization of resistance mediating genes among recent MDR clinical isolates of ST 131 to identify transferable resistance.

Material and Methods:
A collection of clinical *E. coli* isolates was characterized by multi-locus sequence type (MLST) determination using a novel approach based upon next-generation sequencing (NGS). One *E. coli* ST131 isolate with confirmed antibiotic resistance to fluoroquinolones, beta-lactams and aminoglycosides was chosen for conjugation experiments. *E. coli* SG22215 [3] was used as a sensitive recipient strain for detecting all antibiotics of interest. Overnight cultures of both bacterial strains were co-cultivated for four hours at 37°C. Transconjugants were selected for the resistance markers on agar plates containing appropriate antibiotics for selection. Recipient strain identity was verified by phenotypic and genomic markers. Plasmids were isolated and characterized by size electrophoretically. Verification of the resistance plasmids was based upon individual resistance gene confirmation via PCR as well as determining the MIC values for transconjugant, recipient and donor.

Results:
The resistance to beta-lactams and to aminoglycosides was successfully transferred by conjugation. The transconjugants of the sensitive strain with acquired resistance show the presence of large plasmids (>30 kbp). Resistance to beta-lactams and aminoglycosides was cotransferable with one plasmid as indicated by MIC increases from a sensitive to a high-level range.
High-level fluoroquinolone resistance is based on chromosomal target mutations and, thus, was not transferrable.

Conclusion:
The clinical *E. coli* ST131 isolate harbours large plasmids that carry resistance genes for beta-lactams and aminoglycosides, two of the most important groups of antibiotics used for treatment of severe infections. These resistance plasmids can be easily transferred to previously sensitive strains and mediate high-level resistance. This indicates a major future risk for the developing spread of plasmid mediating resistance against essential antibiotic drugs.

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3.4 Cancer

POS.93

Clickable analogues of siderophores as modular ^{89}Zr-chelators for targeted PET imaging

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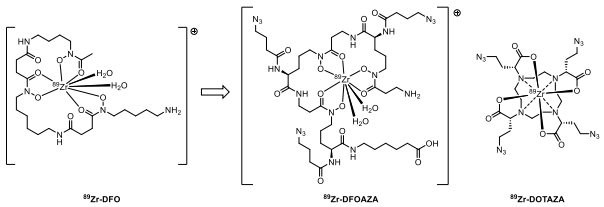
Zirconium-89 is an attractive isotope for diagnostic positron emission tomography (PET) due to cost efficient production, favorable decay characteristics and a half-live of 78.5 h.[1,2] The latter factor makes it an ideal isotope for targeted imaging because it matches the *in vivo* half-life of the most attractive targeting vectors: antibodies and peptides.[3,4] The gold standard for Zr-chelation is desferrioxamine (Desferal®, DFO), a siderophore from the bacteria *Streptomyces pilosus*.[5] Although, DFO is treated as gold standard for Zr(IV)-complexation, it was found to have only limited stability *in vivo* in preclinical studies.
Current challenges for Zr-chelators are the stability of the metal complexes as well as the pharmacokinetics of these substances. We present two new approaches to Zr-chelators that tackle these problems. Our new Zr-chelator, **DFOAZA**, is derived from the natural product DFO and was modified with azide functionalities for click-functionalization. In terms of stability, the cyclic chelator DOTA outperforms all current Zr-chelators.[1,6] We present the first modular DOTA-derived Zr-chelator **DOTAZA**,[7] which can be modified *via* click chemistry or STAUDINGER ligation.



Acknowledgments: National Institute of Health (NIH), Prof. Wolfgang Maison

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POS.94
PST.02

Switching lanes - Metabolic implication of Tigecycline as an efficacious second-line treatment for patients with sorafenib-resistant Hepatocellular Carcinoma

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Introduction: Sorafenib represents the current standard of care for patients with advanced stage hepatocellular carcinoma (HCC) based on two phase III trials that reveal an overall survival benefit of three months compared to placebo [1,2]. Nevertheless, its use is hampered by the frequent occurrence of drug resistance and up to 80% of patients treated with sorafenib suffer from side effects necessitating dose reduction, short-term "drug holidays" or permanent treatment termination [2]. Among all phase III trials that investigated alternative, combinational or second-line treatments to sorafenib exclusively regorafenib was approved for patients that progress during sorafenib therapy. Patients who discontinued sorafenib due to intolerance were not enrolled in the clinical trial, due to the high structure similarity of regorafenib [3,4]. This study investigates the cellular background of sorafenib-resistance and the therapy-limiting rebound growth proliferation reported in patients. We present the clinically approved antibiotic tigecycline as a well-tolerated and powerful second-line treatment for all patients with advanced-stage HCC that acquire resistance to sorafenib.

Methods: To investigate the molecular mechanisms involved in acquired sorafenib-resistance during treatment and after therapy termination we developed a sorafenib-resistant human liver cancer cell line in which we studied proliferation, metabolic parameters as well as mitochondrial integrity and functionality under sorafenib exposure, withdrawal conditions and tigecycline treatment secondary to sorafenib. Cellular alterations on protein and lipid level were investigated by LC-MS/MS-based approaches and the effect on mitochondria was specified by transmission electron microscopy, high resolution respirometry and a glycolytic stress test on the Seahorse XF Analyzer. The therapeutic effectiveness of tigecycline was then proven in vivo by an ectopic tumor mouse model.

Results: Acquired chemoresistance of HCC cells through continuous exposure to sorafenib in a clinical relevant range is accompanied by severe mitochondrial damage resulting in critical dependency towards anaerobic glycolysis. These sorafenib-resistant cells, which lack a functional mitochondrial electron transport chain and fail to maintain their cellular ATP levels, are refractory towards all commonly used chemotherapeutics. Importantly, sorafenib-withdrawal restores mitochondrial integrity and results in a boost of oxidative phosphorylation, ATP production and cellular rebound proliferation. Inhibition of mitochondrial biogenesis by the bacterial translation inhibitor tigecycline decreases the mitochondrial tricarboxylic acid (TCA) - cycle mediated precursor metabolite synthesis by limiting respiratory chain derived reducing equivalents and therefore highly significantly impairs rebound growth after sorafenib treatment termination in vitro and in vivo. This proliferation block under tigecycline treatment can be overcome by exogenous substitution of the respective precursor metabolites, displaying a functionally limiting role of the TCA-cycle activity for rebound growth proliferation of sorafenib-resistant HCC cells.

Conclusion: Antibiotics that inhibit bacterial translation represent not only an attractive second-line treatment for HCC patients, who develop progressive disease during sorafenib therapy, but also for patients who need a treatment interruption or termination due to severe adverse events. As tigecycline is a well-tolerated, clinically approved drug, our study encourages a clinical evaluation of new designation in patients, to prevent tumor growth rebound effects and prolong the life-expectancy of these advanced-stage HCC patients after sorafenib failure.

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POS.95

The insulin-like growth factor 2 mRNA binding protein IMP2/IGF2BP2 is overexpressed and correlates with poor survival in pancreatic cancer

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The insulin-like growth factor 2 (IGF2) mRNA binding protein IMP2 (IGF2BP2) is an oncofetal protein known to be overexpressed in different tumor types. Pancreatic cancer is a very lethal cancer that requires early diagnosis and new treatment options.
The aim of our study was to investigate the role of IMP2 in the initiation and progression of pancreatic ductal adenocarcinoma (PDAC).
IMP2 was significantly overexpressed in a human PDAC cohort (GEO ID: GSE28735) as compared with normal pancreatic tissue (n=45 each; p=9.82e-007). Strict correlation analysis (threshold R^2>0.75) revealed 22 genes highly positively and 9 genes highly negatively correlating with IMP2. Besides genes involved in the inhibition of apoptosis (Bcl-XL R^2=0.79), especially factors involved in ubiquitynation were strongly correlated with IMP2 expression: SMURF1 (R^2= 0.76), FBXO45 (R^2= 0.75). Moreover, protein kinase C (PKC) signaling pathway was distinctly affected: DXS1179E encoding PKC iota R^2=0.77, PKC substrate PLEK2 R^2=0.78, and inositol triphosphate receptor IP3R3 R^2=0.79. Interestingly, IMP2 levels tended to be upregulated also in PanIN lesions (GEO ID: GSE43288), suggesting IMP2 as a marker for early stages of PDAC.
Besides tumor initiation, IMP2 also seemed to have an impact on tumor progression. TGF-beta treatment of Panc-1 pancreatic cancer cells (5 ng/ml for 48 h, n=3; GSE23952) to induce epithelial-mesenchymal transition (EMT) was accompanied by increased IMP2 expression. EMT is important for cancer cells to gain migratory and invasive potential, which is essential for metastasis. Concordantly, circulating tumor cells showed higher IMP2 levels as compared with normal tissue from tumor origin and with normal hematological cells (p=0.0022 both, n=6 each; GSE18670). Accordingly, IMP2 expression correlated with short survival (p=2.41e-05, n=45; GSE28735).
In conclusion, as IMP2 seems to drive tumor initiation and tumor progression of PDAC, it might be an interesting diagnostic and prognostic marker as well as a novel target for the treatment of PDAC.

3.5 Inflammation

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POS.96

The role of the synthetic carbazole derivative C81 during inflammatory processes in endothelial cells

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The endothelium plays a crucial role in chronic inflammatory diseases such as rheumatoid arthritis or atherosclerosis and represents a barrier during the extravasation of leukocytes from blood vessels into the inflamed tissue. The current available pharmacotherapies often show side effects or are of too low efficacy. Therefore, the development of new drugs is an ongoing demand. One lead compound might be the synthetic carbazole derivative C81. A thermal shift assay revealed that the compound inhibits various kinases (e.g. BMP2K, AAK1, CLK1/4) whose biological roles are not yet clarified in endothelial cells. These kinases might play a pathophysiological role in the inflammation-activated endothelium. Therefore, we aimed to investigate the pharmacological potential of C81 during inflammatory processes in the vascular endothelium.

In regard to inflammatory processes, the extravasation of leukocytes was analyzed after treatment of endothelial cells (ECs) with C81. Results of initial experiments demonstrated that the cell viability of human umbilical vein endothelial cells (HUVECs) was not severely affected by C81 concentrations up to 10 µM for 24 h. The compound significantly decreased the adhesion of THP-1 cells (monocytic cell line), peripheral blood monocytes (PBMCs) and primary lymphocytes onto activated HUVECs in vitro. In addition, in vivo results obtained by intravital microscopy of the murine cremaster muscle revealed a significantly reduced adhesion of leukocytes onto endothelial cells after C81 treatment. The surface expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and E-selectin in ECs, which mediate endothelial cell-leukocyte interactions during the adhesion process, were reduced with an IC_{50} value of 5 and 4 µM, respectively. Moreover, the underlying mechanisms of C81 during inflammatory processes were examined. The degradation of IκB-α as well as the nuclear translocation of the NFκB subunit p65 were analyzed after C81 treatment in HUVECs. Western blot analysis indicated that C81 did not influence the degradation of IκB-α in TNF-activated endothelial cells. In addition, neither p65 translocation (immunofluorescence staining) nor NFκB promoter activity (dual luciferase reporter gene assay) were affected by C81. Analyzing the MAPK pathway we found that in C81 pre-treated (10 µM, 12 h) and TNF-activated HUVECs the activation of c-Jun N-terminal kinase (JNK) was markedly reduced. Of note, the mRNA and protein expression of the phosphatase DUSP-1 (dual specificity mitogen-activated protein kinase phosphatase-1), which is able to deactivate JNK, was strongly up-regulated after C81 treatment (qPCR, western blot). Moreover, the analysis of the COX-2 pathway revealed that C81 significantly increased the COX-2 expression in TNF-activated endothelial cells. Interestingly, the secretion of prostaglandins (PGs) was attenuated (western blot, LC-MS/MS), whereas intracellular PG levels remained unaffected.

Our study revealed a promising anti-inflammatory potential of the carbazole derivative C81 in endothelial cells in vitro and in vivo. We will further characterize the effects of C81 on underlying signaling pathways during inflammatory processes in endothelial cells to clarify the compound's mode of action. Furthermore, we will focus on the kinases targeted by C81 in order to elucidate their role in the vascular endothelium.

3.6 Analytics and Toxicology

POS.97

Submission withdrawn.

POS.98

UHPLC-CAD analysis of fatty acids in polysorbate 80: Influence of CAD parameters

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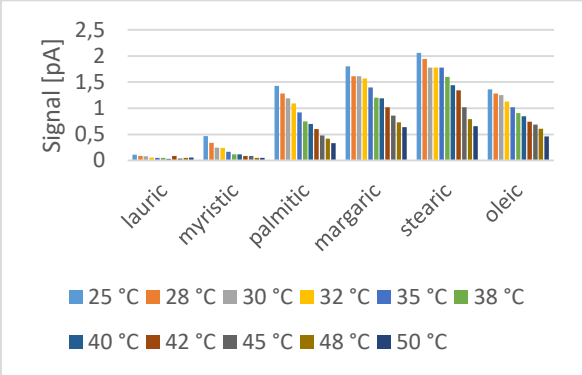
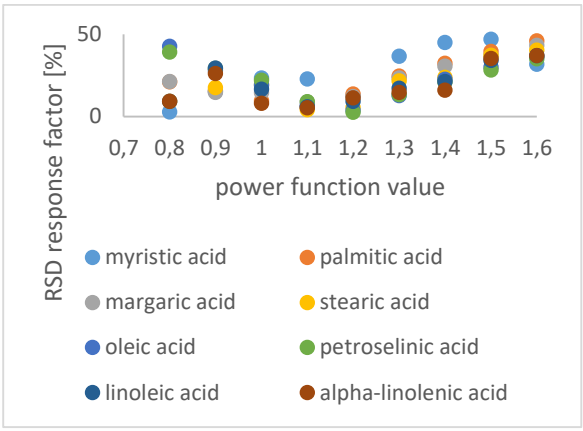
[a]These authors contributed equally to this work.

Polysorbate 80 is an often used excipient in pharmaceutical and cosmetic products. It is also known as Tween 80 and consists of a sorbitan backbone esterified to polyethylene glycols (PEGs) and fatty acids ranging from C_{14} to C_{18}.The analysis of fatty acids by means of HPLC-UV is not possible without additional derivatization or sample preparation steps due to their lack of suitable chromophores. Charged Aerosol Detection (CAD) represents the method of choice in routine analysis for sufficiently non-volatile molecules without chromophores because it is known to be superior to other universal detectors such as the evaporative light scattering detector (ELSD) and because it is cheaper and simpler to use than mass-spectrometry (MS) [1, 2].
Despite its easy-to-use character, CAD parameters have significant impact on the generated signal, which was evaluated in this work. A formerly published HPLC-CAD method [3] was transferred to current generation UHPLC-CAD equipment and influence of the CAD parameters evaporation temperature, power function value (PFV) and filter constant on sensitivity and linearity of the method was investigated systematically. The evaporation temperature was found to be crucial for optimization of sensitivity limits in case of semi volatile analytes (Fig. 1).



Furthermore, the most common linearization approach for calibration curves in CAD analysis consisting of a double-logarithmic transformation was compared to the linearization via adjustment of PFV [4]. Log-log transformation was found to be universally superior to application of an experimentally determined PFV in terms of linearity over two orders of magnitude since the optimal PFV varied for most of the analytes (Fig. 2).



Acknowledgements: University of Würzburg: Christiane Theiss and Oliver Wahl. Thermo Fisher Scientific: Paul H. Gamache and Frank Steiner.

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POS.99

Analysis of Mineral Oil Hydrocarbons for Pharmaceutical purposes using ^{1}H NMR spectroscopy

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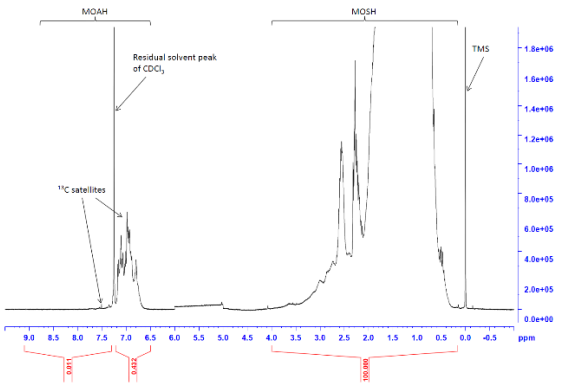
Nowadays there is a widespread use of mineral oil based products like fuels, plasticizers, ink, and lubricants. The initial steps of refinement of crude oil (petroleum) lead to substance mixtures used for fuels like kerosene, gasoline and diesel. Further refinement results in products for pharmaceutical applications e.g. hard, soft and liquid paraffin. Such processes consist of vacuum distillation, solvent extraction, solvent dewaxing, hydrocracking and de-aromatization in order to minimize the content of aromatic structures, which are associated with carcinogenic activity.
In general, mineral oil based substances are complex mixtures consisting of different hydrocarbon molecules of different structures (branched, linear, cyclic), number of carbon atoms, and extent of saturation. These mineral oil hydrocarbons (MOH) can be divided into two main fractions, the mineral oil saturated hydrocarbons (MOSH) and the mineral oil aromatic hydrocarbons (MOAH), which are usually highly alkylated molecules (C_{15} – C_{100}). MOAH can include polycyclic aromatic hydrocarbons (PAH) which are considered to be carcinogenic.
Therefore, the European Pharmacopoeia limits the residual impurities of PAH by measuring a DMSO extract using UV spectroscopy. This purity test is described in the monographs of all sorts of paraffin. Certainly, this test is time-consuming and error-prone. Additionally, the MOAH fraction cannot be detected with this test.
Hence a ^{1}H NMR spectroscopic method was developed for the determination of aromatic traces (including MOAH and PAH) in MOH for pharmaceutical use. All measurements were conducted using a Bruker Avance 400 MHz spectrometer. The evaluation of the MOAH content was performed by calculating the ratio of aromatic protons to aliphatic protons. The technique was tested with several samples collected from different stages of the refinement process. Moreover, a few samples of synthetic paraffin were analyzed. The ^{1}H NMR spectra nicely reflect the purification characteristics of the mineral oil refinement with respect to residual traces of MOAH. Moreover, by evaluating differences in the

degree of aromatic impurities a comparison of a lot of products on the German market was possible.



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POS.100

A novel GCLP-compliant bioanalytical LC-HRMS method for the reliable determination of aldosterone, precursor and metabolite facilitating further insight into paediatric maturation of the renin-angiotensin-aldosterone-system

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Background: Aldosterone is an important marker for end-organ damage and cardiovascular death [1]. While it is intensively investigated in adults, little is known in the regulation of the renin-angiotensin-aldosterone system in cardiovascular diseased children. Since research in children is challenging and still limited, all efforts should be undertaken to enable the maximum data generation during paediatric investigations. Therefore, it appears reasonable to establish a LC high resolution mass spectrometry (LC-HRMS) assay which combines accurate and precise quantification of the analytes of interest, but also allows a deeper insight in paediatric metabolism by non-targeted screening.

Aim: Development of a LC-HRMS bioanalytical assay which enables the robust and reliable determination of aldosterone and its known precursor/metabolite in serum in accordance with international guidelines using a child-appropriate sample volume. Moreover, non-targeted screening by TOF MS scan should be established for a sophisticated investigation of paediatric samples facilitating further insights into the paediatric aldosterone metabolism.

Material and Methods: The determination of aldosterone was conducted with a Sciex TripleTOF 6600 mass spectrometer coupled to a Shimadzu Nexera HPLC-system using a Cortecs C18+ (150x2.1 mm, 2.7µm) analytical column. Data acquisition was conducted by product ion scans for the analytes of interest and non-targeted screening through a TOF MS scan. No artificial aldosterone-free matrix or blood substitutes were used to mimic highest comparability to study samples. Steroid depleted human serum was generated according to an in-house protocol and applied during validation. Sample preparation was done utilizing a tailored HLB Prime 96-well SPE protocol. Validation was conducted in accordance to current EMA and FDA guidelines on bioanalytical method validation [2,3].

Results: Deploying the in-house generated steroid depleted human serum, a bioanalytical LC-HRMS assay encompassing a calibration range of 19.5 - 2500 pg/mL was successfully established utilizing 50 µl of serum. Between-run accuracy showed a deviation from nominal concentration of -1.2% to -7.0% (guideline limit: ±15%; ±20% at LLOQ). Between-run precision was characterised by a CV between 2.1% to 10.2% (guideline limit: ±15%; ±20% at LLOQ). Making the between-run total error ranging from 5.3% to 11.4%. All results complied with the guidelines specifications and conformed its robustness and reliability. Internal standard normalized matrix effect and recovery was investigated in six different human sources. Appropriate short-term/autosampler stability facilitated the investigation of aldosterone and metabolites in a high-throughput 96-well approach.

Conclusion: A low-volume bioanalytical high resolution mass spectrometry assay was successfully developed and validated requiring a minimal child-appropriate sample volume of 50 µl serum. The established calibration range is as wide as to cover known aldosterone concentrations in healthy/diseased adults and children. Moreover, the TOF MS supports the ambition to generate maximum data from the limited paediatric samples and facilitates the possibility of deeper insight on the aldosterone metabolism of children.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602295 (LENA).

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POS.101

Identification of new substrates for the mARC Enzyme System

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The mitochondrial amidoxime reducing component (mARC) is the fourth human molybdenum containing enzyme and identified recently in our lab. The three component system consisting of mARC, cytochrome b_5 and the NADH depending cytochrome b_5 reductase is able to reduce endogenous and xenogenic *N*-hydroxylated compounds [1; 2].

Due to the recent discovery the enzymology has not been investigated in greater detail so far. Until now the detection of possible substrates is performed by HPLC after incubations and work-up procedures. However, the development of a suitable HPLC method for each substrate is very time consuming. Also a variety of interesting potential substrates are not measurable via standard HPLC detectors. A more convenient way of detection is the photometric decrease of NADH. If such an assay is positive a more detailed analysis could follow.

We used a biotransformation assays with recombinant human proteins, performed and compared the turn over rate of the model compound benzamidoxime with new substrates of mARC. In addition, we combined the mass spectrometry with our standard procedure to

identify the reduced forms of these substances. Taking this in account we investigated possible substrates that had not been verified yet.

By this method an impressive number of new substrates were detected and the corresponding transformation are now analysed in detail.

In this context we report the successful identification of hydroxyurea, a well-known drug for chronic myelogenous leukemia, as s mARC substrate with a very high turnover rate. Since it is a long-established drug, the exact metabolism is not accurately recorded yet. Also we can report the Identification of senecivernine *N*-oxide, an metabolite of the toxic pyrrolizidine alkaloide senecivernine. These are some very interesting new discoveries leading into new fields of investigation for the role of mARC in the xenogenic metabolism.

In summary a fast and reproducible method for the detection of new substrates of mARC was developed leading already to so far unknown transformations.

We thank Thomas Behrendt and Sven Wichmann (Christian-Albrechts-University Kiel, Germany) for technical assistance.

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POS.102

Enhancing Inter-Batch Comparability in Untargeted Lipidomics by a Semi-Quantitative Study Design

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With the steadily growing knowledge about lipid metabolism and discoveries concerning their physiological roles in disease progression [1], untargeted lipidomic profiling of biological samples has become a widely used approach for biomarker identification. Most studies focus on the observation of relative fold-changes of detected compounds between control groups and distinct groups of interest. In order to obtain a high degree of comparability, samples of such studies usually require analysis in one continuous sequence with embedded quality controls (QCs). As the method for normalization or the status of the utilized instrument might vary, inter-sequence comparison of samples is difficult to accomplish and extensive validation would be demanded. The ultimate approach to overcome this problem is absolute quantification of compounds of interest. However, accurate determination of absolute levels in complex matrices is difficult to achieve for the hundreds or thousands of features detected in comprehensive analysis due to lack of compound specific calibrants.
In this work we want to present an approach towards quantitative, untargeted lipidomics. For analysis, a fast UHPLC method was applied to obtain lipid separation for precipitated mouse plasma samples. Comprehensive MS and MS/MS data were acquired by a QTOF instrument (TripleTOF 5600+, Sciex) in SWATH (sequential window acquisition of all theoretical fragment-ion mass spectra) mode and data were processed using the MS-DIAL [2] software. For quantification, a lipid mix, consisting of 14 class-specific, deuterated lipid standards, was spiked into pooled matrix as surrogate calibrants and QCs. Identified lipids were quantified via the calibration curve of the respective surrogate calibrant of same or similar class. To increase precision and accuracy, two non-endogenous lipids were uniformly added to all samples as internal standards.
As SWATH yields quantitative data on the MS- as well as on the MS/MS-level, the quality of calibration was compared between signals of TOF-MS, TOF-MS/MS and TOF-MS/MS with deconvolution. However, results are of semi-quantitative nature since the response ratio between target analyte and surrogate calibrant is not investigated for every single identified feature. By determining this ratio for compounds of interest, true absolute quantification can be achieved.

We acknowledge the "Struktur- und Innovationsfonds Baden-Württemberg (SI-BW)" and the German Science Fund DFG for funding Scientific Equipment as part of the DFG´s Major Research Instrumentation Program as per Art. 91b GG (INST 37/821-1 FUGG).

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POS.103
PST.03

Fully validated supercritical-fluid chromatography (SFC) for simultaneous quantification of acidic and basic metabolites of the divisive K^+ channel opener flupirtine

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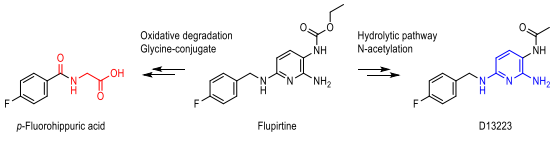
In terms of polarity range, LC covers the widest domain of analytes, but in order to do so, it needs to operate in distinctive separation modes– e.g., reversed-phase, normal-phase, HILIC, ion-chromatography, etc. Paradoxically, this allows for selective analysis of highly similar compounds, while restricting simultaneous analysis of fundamentally different compounds in one single method [1]. These limitations are detrimental to biotransformation studies of lipophilic xenobiotics whose metabolites vary radically in polarity, due to introduction/modification of acidic and basic moieties. Therefore, LC-based metabolomics is either confined to a narrow target polarity range, or requires multiple/2D analytical methods in order to reveal the full metabolic spectrum.

Flupiritine shares the metabolic fate of many lipophilic xenobiotics, to the extent that its hydrolytic and oxidative degradation products vary considerably in polarity and acidity/basicity. It is unique, however, in being the only analgesic that exerts its anodyne effects by targeting K_V7 voltage-gated potassium channels. Due to its unique mechanism of action, flupirtine is clinically as effective as–but does not share the therapeutic limitations of–conventional analgesics such as NSAIDs (GIT-bleeding) and weak opioids (constipation). An increase in severe and fatal liver injury, however, has effectively led to suspension of flupirtine use until the metabolic riddle resulting in hepatotoxicity can be resolved [2].

Here, we present an orthogonal approach to both problems based on supercritical-fluid chromatography (SFC). The target polarity range of SFC is wider than either normal- or reversed-phase LC modes by themselves, due to the versatility of its mobile phase composition (i.e., supercritical CO_2 and polarity modifying additives). This enables simultaneous determination of lipophilic xenobiotics and their hydrophilic metabolites. The technology was applied to flupirtine and two representative polar/acidic and non-polar/basic metabolites in human urine. The resulting method is not only superior in terms of target range, but also an economical and eco-friendly alternative to previous methods. In order to increase the acceptance of SFC, the novel method was fully validated according to EMA-guidelines and applied to clinical samples for proof of suitability [3].



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POS.104

Selective Comprehensive 2D-LC Method for Quality Control of Synthetic (Therapeutic) Peptides

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Peptides have been creating a hugely emerging market for their use as Active Pharmaceutical Ingredients (API), where the majority originate from synthesis. A major problem is that, even with high peak capacities, impurities which are created during the synthesis process may co-elute with the main product due to structural similarities, and thus making them invisible by UV detection. Consequently, a void for quality control has been created, which obviously has to be addressed. Therefore, validated analytical methods, preferably capable of detecting all impurities, are required. The state of the art is to use a reversed phase column (C18) for separation. The aim of this work is to add a second dimension (2D) to the already existing generic peptide separation method (1D). The choice of the stationary phases as a matter of course plays a vital role, as they should exhibit phase chemistries complementary to each other (orthogonality). Aiming at the ampholytic nature of peptides, first a zwitterionic chiral ion-exchanger (Zwix(+)), then a RP column operated at high pH, were integrated as the second dimension. The variation of few mobile phase composition parameters successfully allowed the navigation of the retention behavior of the targeted analyte. In conclusion, our results show that a 2D-LC method can be employed to resolve impurities in synthesized peptides, which cannot be detected using a generic LC method.

Gratefulness is due to Agilent Technologies for financial support through an Agilent Research Award.

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POS.105

Ethanol in medicines for children: Food more relevant than phytomedicines

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Introduction: Liquid dosage forms of phytomedicines are very suitable for children, as they allow the adaption of the dose to the age group. However, as they contain ethanol in many cases, they have been repeatedly triggering critical questions.
Materials and Methods: Therefore, data from non-interventional studies on herbal medicinal products containing ethanol were evaluated. In addition, analytical data for ethanol in food items usual for children were generated by gas chromatography and a scenario for the exposure of a 6 year-old child to ethanol through food intake was generated.
Results and Discussion: When using common herbal medicinal products, an estimation of the ethanol intake for a 6 year-old child reults in a dose of between 70 and 180 mg per single dose. With an intake 3 times daily and related to a body weight (b.w.) of 20 kg, this is 10.1 – 27.0 mg/kg per day [1].
An evaluation of the side effects of these herbal medicinal products was based on non-interventional studies in more than 50.000 children and on spontaneous reports from the use in approximately 3 million children, and did not reveal any ethanol related side effects [2].
For evaluating the uptake of ethanol with food items commonly used in children, ethanol contents in food items were measured [3]. Based on these data, a scenario for the mean ethanol exposure was developed, using data on nutritional habits from USA and Germany. The resulting scenarios lead to an uptake of up to 12.5 – 23.3 mg/kg b.w..

Conclusions: According to these data, the ethanol uptake with herbal medicinal products in children is in an order of magnitude comparable to everyday exposure with usual food items, and therefore there is no cause for toxicological concerns.

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POS.106

Structure-dependent cytotoxicity of different pyrrolizidine alkaloids in primary rat hepatocytes and HepG2 cells: Role of cytochrome P450 3A

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Background: Pyrrolizidine alkaloids (PAs) are secondary metabolites occurring in a wide range of plant species. Some 1,2-unsaturated PAs exert toxic effects through metabolic activation which form the corresponding dehydropyrrolizidine derivatives, primarily in the liver, catalyzed by cytochrome P450 monooxygenases. Due to their hepatotoxicity, genotoxicity and carcinogenicity, the accidental presence of PAs in food, feed and herbal medicinal products can be, depending on the dose, a cause for safety concerns.
Objectives: In order to assess potential risks and confirm the connection between structure and in vitro toxicity [1], we generate data firstly concerning cytotoxicity of some food-relevant PAs. In addition, we also wanted to test in vitro the role CYP3A plays in metabolic activation of PA-induced cytotoxicity.
Methods: After 24 h and 48 h exposure, cytotoxicity of the selected PAs was determined at concentrations ranging from 1 to 300 µM by the Alamar blue assay in primary rat hepatocytes and in the human HepG2 cell line. A kinetic assay analyzing 7-benzyloxyresorufin-O-dealkylation (BROD) was used for measuring the activity of CYP3A enzymes.
Results: A structure dependent cytotoxicity was demonstrated with rat hepatocytes in primary culture. Lasiocarpine, an open-chained di-ester with 7S-structure, proved to be the most cytotoxic followed by the other di-esters echimidine, retrorsine, seneciphylline and senecionine. The mono-esters heliotrine, indicine, europine and lycopsamine were much less cytotoxic. On the contrary, failure to detect cytotoxicity in HepG2 cells is possibly due to the lack of CYP3As. In primary rat hepatocytes, the CYP3A activity decreased rapidly during the culture, therefore, the time to incubation significantly affects the cytotoxicity. These data confirm that CYP3A plays a critical role in PA-induced toxicity.

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3.7 Biotechnology and Biopharmaceutics

POS.107

Aggregation profiling of therapeutic antibodies: Unravelling thermodynamic instabilities

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Monoclonal antibodies (mAbs) are structurally complex multi-domain macromolecules, sensitive to various physical impacts influencing their stability. High antibody stability is an essential requirement for manufacturing, formulation, storage and bioavailability. Predicting long-term stabilities by determining thermal and kinetic properties of mAbs can help to develop optimal antibody candidates with a higher intrinsic stability. The complex thermal unfolding and aggregation behavior of antibodies requires a precise determination of thermodynamic and kinetic parameters providing information about their stabilities. In this study two different label-free thermal techniques (temperature dependent intrinsic fluorescence (IF) spectroscopy, differential scanning calorimetry (DSC)) were used to monitor thermodynamic properties of three different therapeutic mAbs. Thermodynamic parameters, like changes in the van´t Hoff enthalpy (ΔH_{vh}), changes in entropy (ΔS) and differences in the Gibbs free energy change ($\Delta\Delta G$) were derived from an apparent three-state fit function, fitting IF temperature dependent unfolding profiles from the mAbs. The determined thermodynamics from IF fitting were compared to thermodynamics derived from DSC thermograms, showing similar values for both methods. Furthermore the obtained thermodynamic parameters and unfolding profiles were used to establish individual aggregation profile fingerprints of the mAbs, showing predictive character of the determined thermodynamic parameters regarding long-term stability of the therapeutic monoclonal antibodies. Therefore, in-depth unfolding and aggregation profiling is a useful tool for detecting subtle differences in thermodynamic antibody instabilities, and can therefore be used for a directed, tailor-made development of therapeutic antibodies, which possess high intrinsic stabilities.

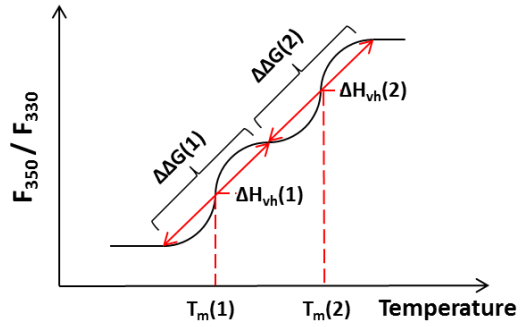

Figure 1: Graphical abstract of an antibody temperature dependent unfolding profile using intrinsic fluorescence spectroscopy. Thermodynamic parameters like ΔH_{vh} and $\Delta\Delta G$ were determined from an apparent three-state fit function applied to experimental unfolding profiles of therapeutic monoclonal antibodies.

POS.108

Predicting Viscosity of Highly Concentrated Therapeutic Protein Formulations

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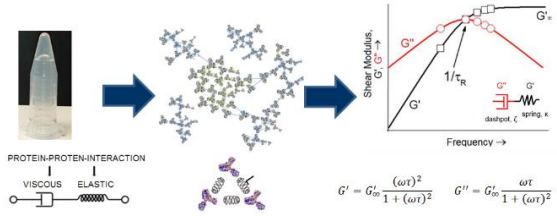
Clinical doses of therapeutic proteins are ranged up to 2mg/kg bodyweigth per patient. Therefore, liquid formulations for subcutaneous application reach concentrations of 200 mg/ml and even higher. Here, one of the most important parameter in the development and application of such highly concentrated liquid formulations (HCLF) of therapeutic biologics is viscosity [1]. Resulting challenges of a drastically increased viscosity are problems in the injectability, a bad patient compliance, high shear stress and reduced protein stability.
In early state development of biopharmaceuticals, where less material is available and the active drug concentration is unknown, forecasting the viscosity of HCLFs at any protein concentration would be a promising feature for risk evaluation in the development process [2]. Here, we demonstrate approaches for the determination of different parameter describing protein-protein interaction, protein hydration, protein conformation at different concentration and the volume fraction of the protein molecule.



Pairwise and higher-order protein-protein interactions, clustering, and protein-excipients interactions are crucial parameters to predict protein-stability and modulate the rheological behaviour. Parameters describing protein-protein self-interaction (e.g. B_{22}, k_D) require dilution of the sample which alters the solution characteristics. However, at high protein concentrations, higher order terms of multi-body interactions contribute to aggregation processes and solution behaviour (shear viscosity) [3]. By studying the behaviour of concentrated protein formulations under high-frequency shear excitation in the MHz range, complex colloid theories can be extended to explain interaction potentials between protein-molecules and high solution viscosity [1].
Besides intermolecular interaction, size, protein conformation and distances between protein molecules in solution also contribute to the solution viscosity. Arrangement of multiple interacting protein molecules lead to different intermolecular distances in solution and altered protein conformations. Additionally, different protein-conformations and distance-distributions were determined within the protein molecules. Correlations to solution viscosity and R_G^{app} contribute to a strong dependency of volume fraction and solution viscosity as predicted by simple colloidal chemistry.

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POS.109

Stabilization of Polysorbate 20 and 80 against oxidation by addition of antioxidants BHT and BHA

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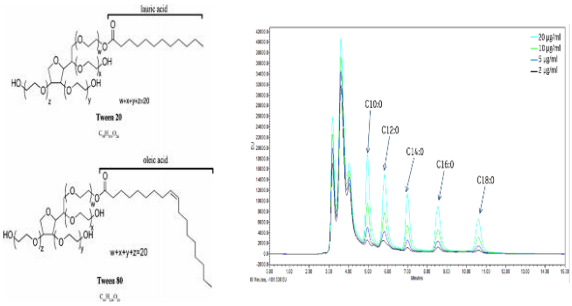
Surfactants, e.g. polysorbate 20 (PS20) and polysorbate 80 (PS80), are used in biopharmaceutical formulations, among other things, to stabilize therapeutic proteins against interfacial stress. PS20 and PS80 - both containing ester and ether linkages - are prone to hydrolytic and oxidative degradation. It has been shown that the oxidative degradation pathway is relevant under pharmaceutical conditions [1]. The purpose of this study was to investigate if oxidation of PS20 and PS80 in aqueous solution can be prevented by the addition of the antioxidants butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA). Therefore, samples (aqueous PS20 and PS80 samples containing either BHT, BHA or no stabilizer) were incubated at elevated temperature (40°C, 75% rH) with air exposure for seven weeks. Afterwards, the following analyses were performed: HPLC fluorescence micelle assay (FMA) to determine remaining concentration of intact (i.e. micelle-forming) polysorbate, ferrous oxidation - xylenol orange (FOX) assay to quantify peroxide level, pH measurement, HS-GC-MS to characterize volatile degradants and LC-UV-MS to determine formation of free fatty acids.

Samples containing either BHT or BHA were better stabilized in both aqueous PS20 and PS80 solutions compared to non-stabilized samples, as seen by higher micelle concentration, lower peroxide levels, stable pH, and lower degree of volatile degradants; the antioxidant levels in the stressed samples were decreased, indicating that indeed the antioxidants were degraded instead of the polysorbates.

It can be concluded that aqueous PS20 and PS80 solutions containing BHT and BHA are more stable, i.e. less susceptible to oxidative degradation, compared to non-stabilized samples.



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POS.110

Polysorbate degradation in formulation development. Will the detection of free fatty acids help to understand the problem better?

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Abstract
Polysorbates (PS) 20 and 80 are the most common non-ionic surfactants used in high concentration protein formulations. Due to their amphipathic nature and hydrophilic-lipophilic balance, they are able to shield the protein against a variety of stress like shear stress, adsorption to surfaces and molecular crowding. Polysorbates are mainly composed of a hydrophilic polyoxyethylene (POE) sorbitan head group and a hydrophobic fatty acid (FA) ester tail. Polysorbate 20 is mainly comprised of POE sorbitan monolaurate whereas for Polysorbate 80 is POE sorbitan monooleate. Although the amount of different fatty acids esters permitted in Polysorbates is strictly defined by the regulatory authorities, the fatty acid composition can differ from batch to batch, along with the ratio of mono, di or polyesters.
During the last decade, PS degradation has arisen as a major quality concern in the biopharmaceutical industry.
For a better understanding of the degradation pathway and the subsequent effect on the protein stability, it is important to characterize and analyse the degradants. One of the speculated degradation pathway of Polysorbates is hydrolysis, which leads to the release of free fatty acids as major degradation product. This work focuses on free fatty acids analysis using PDAM derivatization during chemical hydrolysis of Polysorbates [1].

3.8 Clinical Pharmacy

POS.111

Extracellular nucleotides contribute to the pathogenesis of exacerbations in chronic obstructive pulmonary disease via P2X4- and P2X7-receptor activation

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Background: Chronic obstructive pulmonary disease (COPD) is projected to be the third leading cause of death in the world by 2020 (1) and characterised by fibrosis, emphysema as well as inflammatory processes such as respiratory bronchiolitis and chronic bronchitis. COPD patients are susceptible to bacterial or viral pulmonary infections causing exacerbations (AECOPDs) resulting in episodic aggravation of COPD symptoms, especially airway obstruction. Thus, AECOPD is primarily associated with elevated morbidity, mortality and health care expenses. Under experimental conditions polyinosinic:polycytidylic acid [poly (I:C)] and nontypeable Haemophilus influenzae (NTHI) (2,3) are used to mimic a TLR-3 driven viral exacerbation or a TLR-2 and TLR-4 driven bacterial exacerbation respectively. Purines like adenosine triphosphate (ATP) are commonly known as energy carriers but once in the extracellular space they serve as damage associated molecular patterns (DAMPs) which are capable of modulating immune responses via inducing ionotropic P2X- and metabotropic P2Y-receptor signalling. Recent findings indicate that acute lung inflammations and cigarette smoke exposure are accompanied by elevated ATP levels (4) in the bronchoalveolar lavage fluid (BALF) of mice.
Objective: Investigating the effect of purinergic signalling in COPD and AECOPD pathogenesis.
Methods: Wildtype (C56BL/6N), P2X4 and P2X7-deficient mice were exposed to the smoke of 5 cigarettes on day 1, 7 cigarettes on day 2 and 9 cigarettes on the following two days to induce an acute airway inflammation. To exacerbate the acute inflammation animals received a single intratracheal (i.t.) dose of poly(I:C) (100µg/80µl) on day 3 prior to smoke exposure or $4x10^5$ CFU/80µl (NTHI) on day 4 after the last smoke exposure, respectively. Control animals were exposed to regular room air and received either PBS or one of the two exacerbation stimuli. Mice were sacrificed 24hrs (NTHI) or 36hrs [poly (I:C)] after the final smoke exposure to collect the BALF (bronchoalveolar lavage fluid) for differential cell analysis as well as cytokine measurements and lung pieces for quantitative PCR analysis. In addition, we applied a 4-month lasting chronic approach with a subsequent single NTHI i.t. application and a final fixing step by embedding the lungs in formalin to assess lung emphysema development.
Results: Wildtype mice exposed to cigarette smoke and additionally receiving poly(I:C) / NTHI show an increased *p2rx4* and *p2rx7* lung expression compared to control mice. P2rx4 as well as P2rx7 deficiency were associated with alleviated proinflammatory cytokine and chemokine concentrations in the BALF in terms of IL-1ß, Il-6, TNFα, MIP-1α, IL-17, CXCL-8 and MCP-1. In addition, receptor deficient animals showed reduced neutrophil numbers and diminished concentrations of tissue remodelling enzymes (MMP-9, ELA-2) in the BALF compared to wildtype mice. Subsequent chronic experiments with long term smoke exposure revealed a protection against emphysema development associated with P2RX4 and P2RX7 knockout.
Conclusion: P2X4- and P2X7-signalling contribute to the pathogenesis of exacerbated acute cigarette smoke induced airway inflammation. Thus, targeting P2X4R and P2X7R might reveal new therapeutic options for treating AECOPD. Indicating that antagonist treatment could help to substitute or at least reduce glucocorticoid therapy doses and contribute to increase the quality of life for COPD patients.

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POS.112

Exploring pharmacokinetic/pharmacodynamic relationships of levofloxacin against resistant *Escherichia coli* in a dynamic *in vitro* infection model

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Objectives: Target site concentrations of antibiotics being insufficient to eradicate the pathogen support emergence and spread of antimicrobial resistance. In order to effectively treat infections caused by bacteria with reduced susceptibility and prevent further resistance development it is crucial to understand the relationship between pharmacokinetics (PK) and pharmacodynamics (PD) of the triad antibiotic agent, pathogen and patient. These investigations aimed to characterise the concentration-effect relationship of typical concentration-time profiles (C(t) profiles) resulting from a single levofloxacin (LEV) dose against a resistant *Escherichia coli (E. coli*) isolate in septic patients with different renal functions.

Methods: A previously published population pharmacokinetic model [1] was used to simulate typical C(t) profiles of a septic population resulting from a 750 mg, 90-min intravenous (i.v.) infusion of LEV and to derive the respective experimental parameters for a dynamic *in vitro* infection model (dIVIM). A clinical *E. coli* isolate with a minimal inhibitory concentration (MIC) of 2 µg/mL was exposed to the *in vitro* mimicked C(t) profiles for 24 h (n=3). Samples were taken according to a predefined sampling schedule, LEV was quantified using a validated fluorescence assay [2] and bacterial concentrations over time were determined using a plate counting method. PK/PD indices (AUC/MIC, C_{max}/MIC) and the intensity of antimicrobial effect (I_E) [3] as area between the growth control (GC) curve and time kill curve (TKC) were determined. Additionally, the antibiotic effect at each sampling time was calculated as difference between GC and TKC. Based on experimentally observed LEV C(t) profiles, a compartmental PK analysis was performed to derive LEV PK parameters. Clearance (CL) parameter values were used to determine the corresponding creatinine clearance (CLCR) values based on the covariate effect relation between CLCR and CL estimated in the population PK model [1].

Results: C(t) profiles derived in the dIVIM were within the 90% confidence interval of simulated profiles of septic patients. Experimentally observed and PK model-predicted C_{max} values were similar ranging from 16.2 µg/mL to 17.9 and 16.1 to 16.8 µg/mL, respectively. Predicted AUC values ranged between 77.1 µg·h/mL and 149 µg·h/mL. Thus, corresponding CRCL values of three experimentally observed C(t) profiles represented moderate renal impairment (55.4 mL/min), mild renal impairment (74.7 mL/min) as well as healthy renal function (124 mL/min). In none of the replicates, *in vitro* PK/PD targets (C_{max}/MIC > 10; AUC/MIC > 100 [4]) were reached. Accordingly, regrowth up to a bacterial concentration of 10^4–10^5 colony forming units (CFU) per mL was observable after initial 6 $\log_{10}$-fold reduction of bacterial count. The effect-concentration relationship was characterised by a counterclockwise hysteresis curve. Compared to C_{max} at 1.5 h, the maximum effect (E_{max}) was delayed several hours. LEV concentration was 2 µg/mL at time of E_{max}, which represents the MIC value of the investigated isolate.

Conclusion: *In vitro* mimicked LEV C(t) profiles were in PK range of septic patients with different renal functions. Bacterial regrowth occurred even under exposure to a C(t) profile corresponding to a patient with moderate renal impairment, indicating that therapeutic dosing of 750 mg LEV once daily is not sufficient to treat infections caused by pathogens with reduced LEV susceptibility. Commonly used PK/PD indices and PD parameters, such as C_{max}/MIC, AUC/MIC and I_E and are not sufficient to characterise the PK/PD relationship of LEV, because they do not consider the dimension of time. Equal LEV concentrations can cause different antibiotic effects, depending on previous shape of C(t) profile and duration of exposure. Further investigations are warranted to identify resistance mechanisms, which might emerge under exposure to LEV C(t) profiles, such as upregulation of efflux pumps or alterations of target enzymes, to optimise antibiotic therapy.

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POS.113

A method for the minimally invasive drug monitoring of Mitotane

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Mitotane is the most effective treatment of the adrenocortical carcinoma (ACC), which is a rare neoplasm with a dismal poor prognosis due to its high risk of recurrence even after complete resection and its potential to metastatic spread [1]. Mitotane is used both as an adjuvant treatment after complete tumor resection and for palliative treatment of advanced disease [2]. Although mitotane has been in use for treatment of ACC for decades, many practical issues such as dose-response effects and treatment indication suffer from a poor level of evidence.[3] Mitotane has an extraordinary pharmacokinetic profile. Due to its high lipophilicity, accumulation in fat tissue may lead to elimination half-lives of 18 to 159 days and to a high volume of distribution [3]. Furthermore, it is a substrate as well as an inducer of cytochrome P450 enzymes [3]. Mitotane plasma trough levels within 14 – 20 mg/L are correlated with a higher response rate and a longer survival with acceptable toxicity [4]. However, approximately 30% of the treated patients never attain plasma concentrations in this therapeutic range or the time to reach this therapeutic window is longer than 3 months despite of high dosing regimens (median dose 7.5 g/d) [2]. Accordingly, therapeutic drug monitoring (TDM) is mandatory during the treatment and especially at the initiation of treatment. Frequent home-based therapeutic drug monitoring during the initial phase using microsampling techniques, would be desirable to forecast, whether a patient will reach satisfactory serum concentrations in an acceptable amount of time. Therefore, a simple method enabling the patient to collect capillary blood at home for the control of mitotane blood concentration was developed and validated. MITRA™ VAMS 20 µL micro sampler were used for blood sampling and air dried for at least 12 hours. Afterwards the tip of the MITRA™ VAMS device was removed and the dried blood samples were extracted. Analysis was carried out with a reversed phase HPLC–UV method. During method validation, linearity, precision and accuracy, selectivity, specificity and stability was evaluated. Carry-over effects, recovery and the influence of different haematocrit levels was investigated as well. This new method for the quantification of mitotane from dried blood samples collected at home, combined with model-based simulation could be one way to decide whether a patient should stay on mitotane treatment or not in order to minimize the number of patients receiving this highly toxic substance while having only minimal chances of reaching therapeutic serum levels.

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POS.114

Precision dosing of vancomycin – evaluation of the predictive performance of population models for probabilistic dosing.

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Background: Vancomycin is a frequently used antibiotic in patients suffering from critical infections. Therapeutic drug monitoring (TDM) is required to prevent under dosing or toxic side effects such as nephrotoxicity. To ensure an effective and safe treatment from the start of therapy, probabilistic dosing (pre-tailoring the first dose before TDM) is a useful tool, when an appropriate population pharmacokinetic model is available. The aim of this work was to evaluate published population pharmacokinetic models for vancomycin with regard to their predictive performance for probabilistic dosing, and ultimately select a suitable model for implementation into the TDMx software (www.TDMx.eu).

Method: Eleven population pharmacokinetic models for vancomycin in adults were included in the evaluation after a literature search. The population pharmacokinetic models were recreated and processed in NONMEM® 7.4. As a first step, pharmacokinetic profiles for a standard patient (age: 50 years, body weight: 75 kg, body height: 1.7 m, serum creatinine: 85 µmol/L) were simulated with each model. A dataset of 357 patients (MRSA infection, different degrees of acute kidney injury) was then used to investigate the performance of the models when predicting vancomycin concentrations from patient characteristics. The model predicted concentrations were compared to the observed concentrations and relative bias and relative root mean squared error (rRMSE) were calculated to determine accuracy and precision.

Results: The published models were different regarding the structural model component (5 one-compartment models and 6 two-compartment models) and covariate inclusion. All models utilised patient covariates to describe the pharmacokinetic profile. Highly variable pharmacokinetic parameters were obtained from each model for the standard patient: For one-compartment models, clearance ranged from 2.81 to 7.11 L/h and volume of distribution from 38.2 to 101 L. For the two-compartment models, the parameters were also highly variable (clearance 2.6 - 4.3 L/h, central volume of distribution: 23.5 - 55.5 L, peripheral volume of distribution: 32 - 422 L). The forecasting accuracy in the clinical dataset of 357 patients resulted in a considerably wide range of accuracy and precision with a relative bias ranging from -14.3 to 166.3% and an rRMSE ranging from 62.1 to 200.4%.

Conclusion: Suitable models for probabilistic dosing of vancomycin were identified by evaluation against a clinical dataset. Further investigation of more models is warranted to identify other possible predictive covariates and investigate dose-to-dose forecasting accuracy and precision in order to increase the safety of vancomycin therapy in the treatment of severe infections.

POS.115

Variability of piperacillin serum concentrations in intensive care patients

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Objectives: Piperacillin (PIP) is a broad-spectrum β-lactam antibiotic frequently used for the treatment of severe infections in intensive care patients. Especially in critically ill patients, large variability, due to physiological changes related to their state of disease, is observed [1]. The objective of the present work was to evaluate PIP serum concentrations after standard doses of PIP in a critically ill patient population.
Methods: A monocentric prospective observational study was conducted in an intensive care unit at the University Hospital of Munich. In the study, 60 critically ill patients with severe infections were treated with PIP and Tazobactam (TAZ). According to clinical guidelines and depending on their renal function, the patients received 4 g PIP and 0.5 g TAZ either two (BID) or three (TID) times daily as intravenous 0.5 h short-term infusion. Multiple serum samples were taken and different patient factors were determined. Using R 3.5.0, an exploratory statistical and graphical data analysis was performed. Variability in PIP concentrations was assessed by comparing minimal serum concentrations (C_{min}) among patients. To evaluate if effective PIP concentrations were reached, C_{min} values were compared to non-species-related MIC breakpoints (EUCAST: Susceptible/Intermediate breakpoint: 4 mg/L, Intermediate/Resistant breakpoint: 16 mg/L[2]).
Results: Patient characteristics of the population were highly variable: 17 female and 43 males with a median age of 63.5 years (range: 23-82 years) and a median body weight of 80 kg (range: 50-150 kg) formed the study population. Depending on their renal function, 15 patients were assigned to the BID (renal function < 30 mL/min) and 45 to the TID (renal function > 30 mL/min) dosing regimen. The majority of patients suffered from sepsis (81.7%). Interindividual C_{min} variability was high: C_{min} differed between the 60 patients up to a factor of 1623 (C_{min}: median: 29.8 mg/L range: 0.13 – 211 mg/L), a factor of 112 (C_{min}: median: 46.6 mg/L range: 1.88 – 211 mg/L) within the BID subpopulation and a factor of 1192 (C_{min}: median: 15.4 mg/L range: 0.13 – 155 mg/L) within the TID subpopulation. Intraindividual C_{min} differed up to a factor of 33.8 (median of the factor: 2.33). In the TID dosing subpopulation out of 320 Cmin samples, 71 (22.2%) were below the S/I breakpoint and 155 (51.6%) below the I/R breakpoint. All 108 Cmin samples of the BID subpopulation were above the S/I breakpoint and only 4 (3.70%) below the I/R breakpoint.
Conclusion and Outlook: High variability of PIP concentrations was observed between and within the critically ill patients, with half of the minimum PIP concentrations not reaching the I/R breakpoint in the TID population. The large difference between C_{min} values of the TID and BID population indicates an influence of creatinine clearance on PIP plasma concentrations. Based on drug concentrations in serum a population pharmacokinetic model will be developed to describe the typical pharmacokinetics of PIP and to quantify the different levels of variability. Next a covariate analysis will be performed to determine further patient factors explaining the high variability in the study population and to ultimately identify patients at risk of subtherapeutic PIP concentrations.

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POS.116

Semi-mechanistic modelling of hydrocortisone pharmacokinetics in paediatric patients including dried blood spot data

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Objectives: Patients with congenital adrenal hyperplasia (CAH) have low to no biosynthesis of cortisol and thus require lifelong cortisol substitution. Optimisation of hydrocortisone (HC, synthetic cortisol) therapy in this population is important, since too low or high cortisol concentrations increase the risk of adrenal crisis or Cushing's syndrome [1]. This is challenging, since HC has nonlinear pharmacokinetics (PK) caused by saturable binding to corticosteroid binding globulin, non-saturable binding to albumin and erythrocytes [2] and a dose-dependent oral bioavailability due to saturable absorption [3]. Furthermore, mimicking the physiological concentrations of cortisol is challenging due to its circadian rhythm. Dried blood spot (DBS) sampling is a minimally invasive method to collect samples of very small volumes and is therefore particularly suitable for neonates and infants [4]. Since DBS concentrations are commonly used when monitoring the treatment of CAH patients, it is of high interest to include DBS concentrations into the established paediatric PK models for HC [5]. The objective of this analysis was therefore to exploit the feasibility of using DBS instead of plasma concentrations in the paediatric PK model.
Methods: A developed semi-mechanistic adult PK model for a new HC formulation [6] has previously been reduced to a simplified paediatric model using sparse plasma samples from a phase III study in 24 patients with adrenal insufficiency [7]. Plasma and DBS concentrations of cortisol and the CAH biomarker 17α-hydroxyprogesterone (17-OHP) (n=24 patients), haematocrit (Hct, n=12 patients) from this phase III study, and additional DBS concentrations of cortisol and 16 additional steroids from a follow-up study with the same 24 patients were collected. The relation between plasma and DBS samples of cortisol and 17-OHP was characterised by a graphical evaluation. DBS concentrations were normalised by the Hct to generate a DBS/Hct ratio to account for differences in Hct.
Results: Plasma concentrations of cortisol and 17-OHP were substantially higher than the corresponding DBS concentrations. The plasma/DBS ratio ranged between 2 to 8 and 1 to 15 within and between children for cortisol and 17-OHP, respectively. The nonlinear relation between the cortisol DBS concentrations and cortisol plasma concentrations resembles the nonlinear binding behaviour of cortisol. After normalisation with Hct, the ratio of cortisol plasma concentration and cortisol DBS/Hct was close to 1 in the highest concentration range, whereas for the remaining concentrations and ratios, the nonlinear behaviour remained the same. The relation between plasma concentrations and DBS for 17-OHP was rather linear and the ratio was approximately 4 after taking into account the Hct data.
Conclusion: Our analysis identified substantial differences and high variability between plasma and DBS samples for cortisol and 17-OHP. It was shown, that the main factors for the differences between cortisol plasma concentrations and cortisol DBS data might be Hct and the nonlinear binding behaviour of cortisol. Reasons for the large differences and highly variable ratios need to be further investigated, especially for 17-OHP, in order to enable the inclusion of the DBS data into the paediatric PK model. This model is intended to be used for simulations to evaluate cortisol substitution therapy in order to optimise the HC dosing in paediatric patients.

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POS.117

Dose optimisation of vancomycin in neonates and infants based on a microdialysis feasibility study

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Objectives: Therapeutic Drug Monitoring (TDM) of antibiotic agents in infants and neonates is often used to decrease the risk of therapy failure (underdosing) or adverse drug reactions (overdosing). Considering the drawbacks of conventional blood drawing like discomfort and limited sample volume, suitable alternatives are crucial. This translational study was set up to assess the feasibility of subcutaneous microdialysis (µD) for TDM in infants and neonates, treated with the glycopeptide antibiotic vancomycin (VAN). Previous *in vitro* investigations are required to achieve optimal conditions for clinical µD sampling settings.

Methods: *In vitro* recovery and delivery experiments were performed to identify optimal relative recovery (RR) of VAN in a clinical setting on a paediatric population. Micro- and retrodialysates were sampled at flow rates (FR) of 0.5, 1 and 2 µL/min using three µD catheters (CMA63, 20 kDA cut-off) fixed in a standardised *in vitro* microdialysis system. A reported pH dependency on the RR of VAN [1] was studied by investigating different perfusates as Ringer's solution (unbuffered) and phosphate buffered saline (PBS) with pH values of 7.0 und 7.4 to evaluate the influence of changing pH values, which may also occur in septic neonatal patients. Feasibility of clinical *in vivo* µD was evaluated in nine infants and neonates being treated with VAN following standard dosing regimens based on weight and age [2]. µD samples were taken every 30 min over a period of 24 h. Retrodialysis was performed for catheter calibration using 10 times the expected minimum plasma concentrations of VAN. µD samples were analysed using LC-MS/MS (Agilent 1290 Infinity II LC system, triple quadrupole MS/MS system) to obtain individual VAN concentration-time profiles.

Results: Using previous *in vitro* optimised perfusate of Ringer's solution and phosphate buffered saline at a flow rate of 1 µL/min and a pH value between 7.0 – 7.4, *in vivo* µD settings were optimised. Indeed, as RR was comparable for delivery and recovery of VAN, retrodialysis could be used for *in vivo* catheter calibration. Subcutaneous µD was well tolerated by all patients (gestational age: 28 – 63 weeks, weight: 890 – 5890 g); no adverse events to subcutaneous µD were noted. Using the developed LC-MS/MS method, VAN was successfully quantified in the dialysate samples.

Conclusion: *In vitro* characterisation of the analyte prior to *in vivo* µD ensures optimal sampling, allowing for maximal knowledge generation. Subcutaneous µD is applicable for the determination of individual concentration-time profiles in neonatal patients. Pharmacokinetic analysis of the obtained target-site concentrations can be used for guidelines towards TDM and improve dose recommendations of VAN in neonates and infants.

Further investigations to identify optimal *in vitro* µD conditions by mimicking *in vivo* settings improve better understanding and aid in designing and optimising new clinical studies.

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POS.118
PST.04

An *in vivo-in silico* approach to assess the pharmacokinetics of a novel concept combining amoxicillin with an immunomodulatory drug.

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Background: Emergence of bacterial resistance has become a major threat in healthcare, in particular in lower respiratory tract infections as a leading cause of death worldwide [1]. To address this increasingly important challenge, the pursuit of novel approaches is essential. One strategy is the use of immunomodulatory drugs in combination with antibiotics, which might contribute to minimising both emergence of antibiotic resistance in pathogens and treatment failure. In this study, we aimed to assess the pharmacokinetics (PK) of the antibiotic drug amoxicillin (AMX) as monotherapy and in combination with a toll-like receptor 4 (TLR4) agonist for the treatment of pneumococcal pneumonia.
Methods: Female outbred Swiss/CD1 mice, either healthy or infected intranasally with *Streptococcus pneumoniae* serotype 1, were treated intraperitoneally with monophosphoryl lipid A (MPLA, 50 µg/mouse), intragastrically by oral gavage at low and high doses of AMX (10 µg/mouse or 350 µg/mouse, respectively) or with a combination of AMX and MPLA. Per mouse, 1-2 blood samples were taken at pre-defined time points (0, 0.17, 0.5, 1, 2, 3, 6 h). AMX concentrations over time were determined in serum by an LC-MS/MS assay, which was developed and validated according to EMA guidelines [2]. Nonlinear mixed-effects modelling was then performed to investigate the PK of AMX using NONMEM® (7.4.1).
Results: An LC-MS/MS assay was successfully developed, validated and applied to the serum study samples by only requiring 10 µL of mouse serum. The calibration function covered a broad concentration range of expected serum concentrations (0.01-10 µg/mL) and a lower limit of quantification (LLOQ) of 0.01 µg/mL was determined. A 2-compartment model with first-order absorption and linear elimination best described the PK of AMX. The murine infection status was not a significant covariate on PK parameters, whereas co-administration of MPLA was identified as covariate on AMX clearance (0.6-fold decrease of clearance by combined treatment).
Conclusion: For quantification of AMX in small volumes of mouse serum a rapid, simple and sensitive LC-MS/MS method was developed. The determined PK interaction between AMX and MPLA demonstrated an influence on AMX clearance. This indicates that antibiotic drug interactions require to be evaluated both on the level of PK as well as pharmacodynamics (PD). Hence, additional PK/PD modelling is warranted to link the obtained results of PK and PD experiments (bacterial load, survival). In a final step, *in vitro* and *in vivo* results shall be used to translate them into a clinical setting.

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POS.119

Population PK/PD analysis suggests reduction of dosing interval for infliximab in inflammatory bowel diseases

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Background: The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are chronic diseases affecting the digestive tract and the colon, respectively. Patients typically experience diarrhoea up to the level of incontinence, abdominal pain, rectal bleeding, as well as extraintestinal symptoms, overall causing a poor quality of life [1]. Clinically, IBD are characterised either by a relapsing course of disease or a chronic disease activity. The pathogenesis derives from a dysregulated intestinal mucosal response against commensal gut flora, partially in genetically disposed individuals, which is leading to chronic inflammation of intestinal mucosa for which tumour necrosis factor alpha (TNFα) is a key pro-inflammatory cytokine [2]. Thus, monoclonal antibodies targeting TNFα proved themselves to be a promising therapy for IBD patients [3]. However, infliximab (and anti-TNFα in general) therapy is facing challenges as many patients (up to 60 %) lose response to the drug over time [4]. Pharmacometric approaches offer a tool to understand the underlying processes and improve the therapy success. The aim of this study was to quantitatively characterise pharmacokinetics (PK) and pharmacodynamics (PD) of IFX in IBD and exploit the resulting PK/PD model to assess standard dosing regimen.

Methods: Clinical data for this analysis originated from patients ($n_{patients}$ = 121) with IBD treated at the outpatient clinic of the Medical University of Vienna and were collected as part of an investigator initiated trial. The patients were on maintenance therapy with IFX and received 2-h IFX infusions of absolute doses between 100 and 1300 mg. As part of therapeutic drug monitoring blood samples were taken at mid-term and at end of a dosing interval and concentration of IFX ($n_{PK\ observations}$ = 388) and C-reactive protein (CRP) as a disease activity marker ($n_{PD\ observations}$ = 339) were measured. In addition, information about potentially influential covariates (concentration of anti-drug antibodies (ADAs), serum albumin, co-medication, etc.) was collected. To analyse the data, nonlinear mixed effects modelling approach was applied using NONMEM (version 7.3.0), PsN and Pirana for modelling and R for pre- and post-processing.

Results: First, a PK model describing the time course of IFX in the IBD patients was developed: A 2-compartment disposition model with linear elimination described the data well. As next step of the PK model development effect, numerous patient- and disease-related characteristics on the exposure (i.e. clearance, CL) of IFX were investigated in order to identify patient subpopulations at risk of under-exposure. The resultsindicated that patients with (1) higher disease activity, (2) higher body weight, in whom (3) ADAs have developed and/or (4) who were co-treated with immunomodulators have increased IFX clearance. Based on the mechanism of action of IFX, the PK model was extended to incorporate PD data (i.e. CRP concentration), enabling characterisation of exposure-response relationship. The PK/PD model was used to assess the standard IFX dosing regimen in terms of suppression of CRP production and suggest alternative dosing regimens.

Conclusions: This analysis yielded a PK/PD model that successfully described exposure-response relationship of IFX in IBD patients. Moreover, patient subpopulations at risk of under-exposure were identified. Furthermore, dosing regimens for these subgroups that lead to higher IFX exposure compared to standard were identified.

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POS.120
PST.05

Minimally invasive, model informed drug monitoring of tyrosine kinase inhibitors

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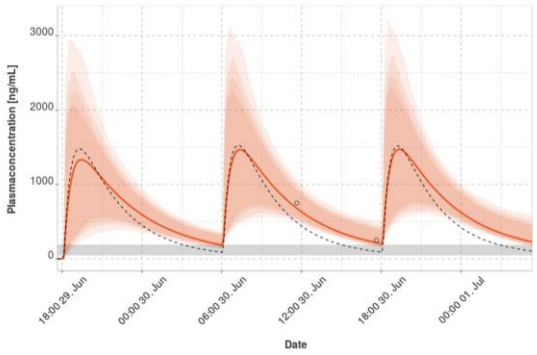
In the last two decades, therapy of oncological diseases has changed to a more target orientated treatment with kinase inhibitors. However, tyrosine kinase inhibitors are associated with a high rate of adverse effects. Mostly, the success of treatment highly correlates with undesirable side effects leading to a lower quality of life and a reduced adherence, which might even end up in a cessation of treatment [1]. For some substances, like the group of antiepileptics, therapeutic drug monitoring already is a well-established method, to monitor plasma levels and to adapt the therapy. Until now, there is little recommendation and experience with therapeutic drug monitoring of tyrosine kinase inhibitors. The pharmacokinetics of most TKI exhibit a high interindividual variability [2]. Depending on the drug substance, various factors have an impact on absorption, distribution, metabolism and elimination. Furthermore, a correlation between dose and side effects was published for most of the substances. Therefore, therapeutic drug monitoring of tyrosine kinase inhibitors could optimize the therapy and reduce side effects [3,4]. In order to early identify risk groups for under- or overdosing, we developed a web-based application for the blood level simulation of Dabrafenib using a published population PK-Model [5] [https://pkmetrix.shinyapps.io/Dabra_v2].



The Model is used to describe the status of blood level according to dose, therapy duration and covariates such as sex, and body weight. With this application, it is possible to identify patients at risk even without monitoring the drug concentration. Furthermore, we are developing minimal invasive methods for capillary blood sampling using the MITRA VAMS device combined with HPLC-MS/MS and online solid phase extraction. This technique would also enable patients and clinicians to perform therapeutic drug monitoring of TKI in a more convenient way. Patients collect the samples and send them to the laboratory themselves, thus there would be no need for a consultation at the hospital for blood or plasma collection purposes, which in turn could, apart from minimizing stress for the patients, also reduce treatment costs. Independent collections of multiple blood samples at different times, combined with the application gives the opportunity to individualize the therapy and optimize the treatment.

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POS.121

Evaluation of RAAS peptides in complex biological matrices in paediatric patients: Development and validation of a low-volume LC-HRMS Method

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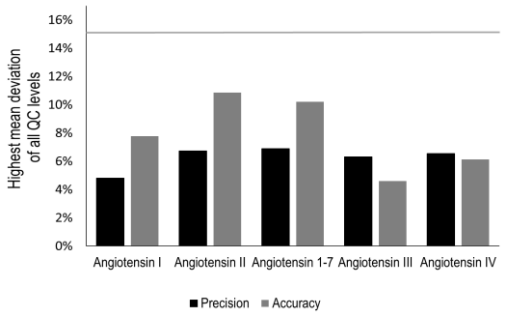
Introduction: The renin-angiotensin-aldosterone system (RAAS) plays a key role in the progression of cardiac and renal failure. While inhibition of enzymes/receptors in context of renin, angiotensin I, angiotensin II and aldosterone found their way into guideline-compliant drug therapy, new potential target structure within the RAAS are continuously identified and provide further insight into the RAAS in adults [1]. These efforts resulted in more than 20 drugs being approved for heart failure in adults, while no drug is approved for paediatric heart failure. The latter emphasises lacking data for rational treatment in children. Appropriate bioanalytical assays would facilitate current ambitions to investigate the maturating RAAS as comprehensively as in adults. Thus, a liquid chromatography high resolution mass spectrometry (LC-HRMS) method was developed, enabling the comprehensive determination of known (angiotensin I and II) and recently focused peptides in adults (angiotensin III, IV and 1-7) also in children.

Materials and Methods: Shimadzu Nexera LC-system coupled with a high resolution mass spectrometer (TripleTOF 6600, Sciex) was utilized to simultaneously investigate angiotensin I, II, III, IV and 1-7 in small plasma volume applicable to paediatric research. Data acquisition was conducted by product ion scans for the peptides of interest and non-targeted screening through TOF MS scan. Chromatographic separation was achieved on a XBridge® BEH C18 column (2.5 µm, 4.6x150 mm) applying a gradient of water (0.1% FA) and methanol (0.1% FA) with DMSO as additive. To preserve the enzymatic degeneration, samples were spiked with an inhibitor cocktail and cooling conditions were applied prior to 96 well µelution solid-phase extraction. The bioanalytical method validation was conducted based on international guidelines [2,3].

Results: The established sample preparation and bioanalytical LC-HRMS assay allowed for the reliable determination of angiotensin I, II, III, IV and 1-7. The linear calibration ranges were successfully established from 7.83 pg/mL to 1000 pg/mL for all peptides of interest. Obtained between-run accuracy and precision values were well within international guideline limits (Figure 1) by applying 50 µL plasma. Within-run accuracy varied from 1% to 11%, while within-run precision ranged from 1% to 12% (guideline limits: ± 15%; except at LLOQ: ± 20%). The maximum total error of the high resolution targeted peptide quantification ranged from 12% for angiotensin I to 24% for angiotensin 1-7. Furthermore, internal standard matrix effect and recovery were investigated in six different human donors to prove robustness and reliablility across different sources. The established non-targeted screening by TOF MS ensured maximum data extraction while the developed high trough put approach the method's applicability in upcoming paediatric clinical trials.



Conclusion: The FDA/EMA-compliant bioanalytical LC-HRMS assay accurately and precisely quantified all peptides of interest in 50 µL plasma. It is useful for GCLP-compliant clinical studies and enables sophisticated investigations in children. Moreover, the TOF MS supports the ambition to generate maximum data from the limited paediatric samples and facilitates the possibility of deeper insight on the maturating RAAS.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602295 (LENA).

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POS.122

A combined pharmacometric-whole body physiologically-based pharmacokinetic PK (WBPBPK) approach to understand the pharmacokinetics of moxifloxacin

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Background: Suboptimal tissue site exposure is a critical factor for therapeutic failure and potentially stimulating antibiotic resistance. The aim of this study was to develop a combined pharmacometric WBPBPK model to describe tissue site pharmacokinetics of moxifloxacin in patients with sepsis.
Methods: Plasma, subcutaneous and muscle tissue samples from 10 patients were collected at day 1, 3 and 5 after intravenous once daily dosing of 400 mg moxifloxacin. Microdialysis was used to collect samples from subcutaneous and muscle tissue. Ultrafiltration was used to determine the unbound moxifloxacin concentrations in plasma. In the first step, an integrated empiric microdialysis pharmacometric model [1], which incorporates estimation of recovery measurements for microdialysis data was developed using a nonlinear mixed effects approach. In the next step, to get a more physiologic understanding of the moxifloxacin pharmacokinetics, a WBPBPK model was built to describe the concentration time profile in different tissues. Blood flows and tissue volumes were calculated from the ICRP report [2], scaled by sex and body weight. Tissue partition coefficients (KP_{UU}) relating unbound tissue concentrations to unbound plasma concentrations of moxifloxacin were calculated based upon their physicochemical properties [3] and used as informative priors during estimation from the available clinical data. NONMEM® 7.4 was used for all estimation and simulation tasks.
Results: For the empiric approach, a four compartment model with total body weight as a covariate on clearance (CL) and central volume of distribution (V1) best described the plasma pharmacokinetics of moxifloxacin. For a 75 kg patient, CL was 12.8 L/hr (interindividual variability, IIV: 28 %CV), V1 was 94.2 L (IIV: 15.2 %) and peripheral volume of distribution (V2) was 81.1L (IIV: 103 %). The fraction of unbound drug in plasma was 0.76 (IIV: 5.9%). Although the model was able to describe all observed data, estimation of the distribution volumes of the tissue compartments was not possible, which did not allow for a mechanistic interpretation. Conversely, the WBPBPK model consisting of 15 physiologically-motivated compartments allowed for a meaningful

physiologic understanding of the distribution spaces of moxifloxacin and successfully predicted the concentration time profiles of plasma and the tissues. The typical KP_{UU} for skin and muscle compartment was estimated to 0.86 and 0.99, respectively.
Conclusion: Moxifloxacin displayed good tissue penetration and the unbound concentrations in muscle and skin tissue approximated unbound plasma concentrations. The pharmacometric WBPBPK combined modelling approach described the moxifloxacin pharmacokinetics in a more mechanistic way compared to the empiric model. In a next step, the WBPBPK model will be linked to pharmacodynamic time-kill curve studies to predict the bacterial killing at the target tissues and to optimise therapy to overcome resistance development.

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POS.123

Pharmacometrics-based optimisation of antiinfective therapy exploiting target-site pharmacokinetics assessed by microdialysis – opportunities and case study with cefuroxime

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Background and objectives: An adequate unbound antibiotic concentration-time profile at the target site of an infection is crucial for effective prophylaxis or therapy. However, often information about target-site pharmacokinetics (PK), especially drug distribution to the site of bacterial infection, are lacking. Microdialysis is a minimally invasive, highly sensitive technique to assess the pharmacologically effective (i.e. unbound) concentrations of analytes in various extracellular fluids, such as (i) plasma, (ii) interstitial fluid (muscle, adipose tissue) or (iii) transcellular fluid (brain, joint) [1]. Hence, microdialysis sampling at the assumed target site of an infection can contribute to further elucidate PK properties of the antibiotic of interest. Using this data in combination with the nonlinear mixed-effects (NLME) modelling approach, the understanding of drug disposition at the potential target site can be further enhanced [2]. The herein presented case study aimed at evaluating the target-site PK of cefuroxime (CEF), a broad-spectrum cephalosporin antibiotic, after perioperative standard dosing in different matrices, including synovial fluid of the knee (SF_{knee}) and interstitial fluid of muscle tissue (IF_{muscle}) assessed by microdialysis. The objective was to establish a PK model for all matrices, combining microdialysis and the NLME modelling approach, to characterise CEF distribution and evaluate the current dosing regimen in terms of prophylaxis or treatment of septic arthritis.

Methods: A PK pharmacometrics analysis was performed with data from an open-labelled, single-centre Phase I microdialysis study (EudraCT: 2012-000379-18) [3]. Patients (n=10, 8 male, age: 18.7–61.7 years, weight: 58.0–118 kg) undergoing elective knee arthroscopy received a single postoperative infusion of 1.5 g CEF over 30 min. In addition to plasma sampling, microdialysis was performed in the synovial space of the knee and in the skeletal muscle of the thigh, using retrodialysis for catheter calibration. For each measurement matrix (plasma, SF_{knee}, IF_{muscle}), samples were collected pre-dose ($n_{median/matrix}$=10) and every 30 to 60 min up to 8 h after dosing ($n_{median/matrix}$=110). Data from all matrices was simultaneously analysed using R (3.5.0) and NONMEM (7.3.0). To evaluate the current dosing regimen, probability of target-attainment in plasma (65% time of unbound CEF concentrations above the minimum inhibitory concentration, $fT_{>MIC}$) was assessed via Monte-Carlo simulations.

Results: Microdialysis was successfully conducted in the synovial space of the knee and in the skeletal muscle of the thigh. A PK model approximating the body to two compartments, representing (i) systemic circulation and (ii) peripheral space, described the data best. Estimated parameters (linear CEF clearance, volumes of the two compartments and CEF exchange between the compartments) were physiologically plausible and in the range of previously reported values [4]. Due to delayed, but kinetically similar, distribution characteristics of CEF into the SF_{knee} and IF_{muscle}, both matrices were assigned to the peripheral compartment. To account for the extent of CEF distribution into SF_{knee} and IF_{muscle} in the model, tissue distribution factors were estimated. Overall, CEF displayed good penetration abilities into both SF_{knee} and IF_{muscle}, with respect to peripheral concentrations. The probability of target-attainment >90% in plasma was ensured for pathogens with MIC values up to 1 mg/mL, e.g. representing the most prevalent MIC for septic arthritis-causing *Staphylococcus aureus*.

Conclusions: Microdialysis represents a highly valuable method to assess target-site PK of antiinfectives. Combining microdialysis and the NLME modelling approach, a pharmacometric model simultaneously describing the PK of CEF in SF_{knee}, IF_{muscle} and plasma after single infusion in patients undergoing knee arthroscopy was successfully developed and target-attainment in plasma evaluated. In a next step, potential factors influencing the PK (covariates) shall be investigated and the final model shall be used to assess target-attainment of the current dosing regimen in SF_{knee} and IF_{muscle}.

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POS.124

Evaluation of Tissue Analytic in Combination with PBPK Modeling

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Despite existing guidelines and recommendations, there are important issues of uncertainty regarding the timing and dose of perioperative prophylaxis (PAP). The amount of tissue penetration of the antibiotics used for PAP is often unclear. The goal of this study is to assess and predict the plasma and tissue concentrations of cefuroxime used for PAP. We collected plasma and lung tissue samples from 25 patients receiving cefuroxime for PAP.

For tissue homogenization, we used dry ice and a disposable grinder. In a next step, we dried the received product under vacuum. Cefuroxime was extracted from the powder by using a mixture of acetonitrile/chloroform. After centrifugation, the supernatant was injected into HPLC system with UV-detection. To take the blood

contamination into account we used the three wavelength method described by Harboe [1], for free haemoglobin measuring. Furthermore, the extraction recovery and loss of water during the drying process were specified and considered as correction factors.

For drugs with mainly extracellularly distribution, such as beta-lactams, grinding up the tissue means dilution of the drug by mixing intracellular and extracellular fluids, resulting in underestimation of its concentrations at the site of infection [2]. Physiologically-based pharmacokinetic (PBPK) modelling (PK-Sim®/MoBi® [3]) allows to predict intracellular and extracellular concentrations.

We used our PBPK model [4] for simulations and a scale-up from the fitted tissue to interstitial concentrations. Alternative dosing regimens for PAP in varying populations were simulated to develop more precise dosing recommendations

For *Staphylococcus aureus*, one of the most relevant pathogen in surgical site infections, adequate interstitial unbound concentration were reached with a dose of 1.5 g given every 2.5 h. With less sensitive bacteria such as especially *Escherichia coli,* the dose regimes do not lead to sufficient interstitial unbound concentrations in most populations.

Our results show that the use of a PBPK software is possible to describe tissue concentrations adequately (MPE = 0.4%; MAPE = 34.5%). Moreover we established a simple and cheap method to quantify tissue concentrations. Using our established model we were able to recommend a more adequate dosing regimen for cefuroxime.

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POS.125

Posaconazole Pharmacokinetics and Dose-Response Relationship for Invasive Aspergillosis, Reflected by Suppression of the Galactomannan Index

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Objectives

Posaconazole is used in treatment and prophylaxis of invasive aspergillosis.

To monitor pharmacodynamics of antifungal substances in-vivo, it has been shown, that the polysaccharide galactomannan is a valid PD surrogate regarding aspergillosis. Although current guidelines suggest therapeutic drug monitoring to ensure adequate posaconazole exposure, there is some uncertainty about therapeutic targets for both prophylaxis and treatment of an established disease.

Methods

In 46 persistently neutropenic rabbits, the PKPD of posaconazole was investigated for the treatment and prophylaxis of invasive pulmonary aspergillosis due to *Aspergillus fumigatus* [1].

Antifungal therapy consisted of posaconazole at 2, 6, and 20 mg/kg of body weight, orally. To evaluate the pharmacodynamics, galactomannan Index (GAI) was collected every other day during the study. Nonparametric PKPD model building was performed using the Pmetrics Package in R [2]. Consequently, the final PKPD model was used to depict the dose-response relationship of posaconazole and the GAI.

Results

A one-compartment model with first-order absorption from a depot compartment and linear elimination best described the pharmacokinetics of posaconazole.

The pharmacodynamic effect of posaconazole plasma concentrations on GAI was best described by dynamic Hill-functions reflecting growth and kill of the fungus.

Through simulations of $AUC_{0\text{-}24h}$ at steady-state, the dose-response relationship between posaconazole and the GAI was explored. The relationship can be described by the exponential function:

$$AUC_{GAI\ 0\text{-}24h} = 104 * e^{-0.07 * AUCposaconazole\ 0\text{-}24h} + 17.61$$

Conclusion

The nonparametric population PKPD model adequately describes posaconazole pharmacokinetics and its pharmacodynamic effect on fungal growth, reflected by the GAI in both treatment and prophylaxis of invasive aspergillosis. An exponential decline best described the dose-response relationship between posaconazole plasma concentrations and the GAI.

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POS.126

Physiologically-based Pharmacokinetic Modelling of Risperidone and its Active Metabolite 9-Hydroxyrisperidone in Subjects Genotyped for Cytochrome P450 2D6

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BACKGROUND AND OBJECTIVES: Physiologically-based pharmacokinetic (PBPK) modelling provides a powerful tool to predict plasma concentrations for individual patients or a population. This could be very useful in psychotropic drugs such as risperidone showing a close relationship between plasma concentration and its pharmacological action. Regarding the drug metabolism, risperidone exhibits a high interindividual variability triggered by the metabolizing cytochrome P 450 (CYP) 2D6 enzymes [1, 2]. The aim of the project is to develop a PBPK model for risperidone and 9-hydroxyrisperidone, by taking into account the CYP 2D6 genetic polymorphism.

METHODS: Risperidone's and 9-hydroxyrisperidone's plasma concentrations were available after oral single dose administration from 71 healthy volunteers. According to CYP 2D6 genotype, subjects were classified in four phenotypes [3]: extensive metabolizer (EM, n=33), intermediate metabolizer (IM, n=26), poor metabolizer (PM, n=6) and ultra-rapid metabolizer (UM, n=6). Literature knowledge about risperidone, 9-hydroxyrisperidone and physiological changes according to the different CYP 2D6 genotypes was used for model development using the software PK-Sim® as part of the Open Systems Pharmacology Suite [4, 5]. In addition to CYP 2D6, the metabolizing enzyme CYP 3A4 as well as the P-glycoprotein transporter were integrated into the model.

RESULTS: The PBPK model for risperidone and 9-hydroxyrisperidone can account for all types of CYP 2D6 metabolizer: EM, IM, PM and UM. Mean prediction error (MPE) and mean absolute prediction error (MAPE) of risperidone were 52.2 % and 54.2 % for EM's, 37.4 % and 43.6 % for IM's, 28.0 % and 49.4 % for PM's and 45.4 % and 62.6 % for UM's. MPE and MAPE of 9-hydroxyrisperidone amount -22.2 % and 22.7 % for EM's, -10.6 % and 18.7 % for IM's, -7.80 % and 15.4 % for PM's and -28.3 % and 28.3 % for UM's. After single oral administration of 1 mg risperidone, a clear association between the number of active alleles and the pharmacokinetic parameters for risperidone and 9-hydroxyrisperidone was observed within the first 96 hours after intake. The average clearance were 5.5 ml/min/kg, 2.6 ml/min/kg, 1.2 ml/min/kg and 6.8 ml/min/kg for EM's, IM's, PM's and UM's, respectively.

CONCLUSION: The newly developed PBPK models for risperidone accurately describe risperidone's and 9-hydroxyrisperidone's plasma concentrations after oral single dose administration for all four CYP 2D6 phenotypes. In the future, PBPK models could be used as a valuable tool to draw conclusions about the existing genotype without previous genotyping as well as a cause for dose adjustments of risperidone during therapy.

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POS.127

Quantification of Pharmacokinetic Interactions: SCHOLZ Datenbank's Multi Drug Drug Interaction Calculator vs. Physiologically Based Pharmacokinetic Modeling

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INTRODUCTION: Nowadays, polymedication plays an immense role both in clinical and ambulatory care. Patients are treated with drugs, which are influencing each other, so their plasma concentrations may increase or decrease. Especially the inhibition of liver enzymes like cytochrome P450 (CYP) oxygenases provokes a higher risk for side effects. So, there is a great need to predict the possible extent of a pharmacokinetic interaction e.g. if a dose reduction is useful when those drugs are combined.
OBJECTIVE: SCHOLZ Datenbank's multi drug drug interaction (MDDI) calculator allows the prediction how far an inhibitoric drug influences another drug's area under the curve (AUC/exposure). To evaluate the software's prediction ability, physiologically based pharmacokinetic (PBPK) modeling via PK-Sim® is used.
METHODS: A therapeutic drug monitoring study, including 71 elderly patients was executed. Samples were taken via dried blood spot method. The plasma concentrations of four psychopharmaceutics - mirtazapine, risperidone and citalopram and its eutomer escitalopram - were measured by using LC-MS [1]. To detect the potential pharmacokinetic interactions, the subjects' medication plans were analysed with the software SCHOLZ Datenbank. The most frequently displayed interaction was between the tetracyclic antidepressant mirtazapine and the noradrenaline and serotonin reuptake inhibitor venlafaxine (n=14).
Simple substance models for mirtazapine and venlafaxine were created including their physico- and biochemical properties. The models were evaluated with literature values. Afterwards, the PBPK models for both drugs were combined in an interaction simulation.
RESULTS: In contrast to the PBPK-model, where no difference in the AUC of the major CYP 2D6 substrate mirtazapine – potentially caused by the weak CYP 2D6 inhibitor venlafaxine [2] - was stated, SCHOLZ Datenbank's MDDI calculator predicts an 8 % higher exposure. This effect caused by the venlafaxine is, however, consistent with clinical data measured in scenarios with CYP 2D6 Poor Metabolizers mimicking drug drug interactions through strong CYP 2D6 enzyme inhibitors [3, 4].
CONCLUSION: To evaluate the model's clinical relevance more clinical research and comparison is needed in conjunction with other CYP 2D6 substrates and inhibitors. Also, research should be extended to other pharmacokinetic interactions.

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POS.128

Detection of Factors for Successful and Continuous Implementation of Medication Reviews in Community Pharmacies

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Background: Medication reviews (MR) are a valuable tool to improve medication safety and appropriateness especially for multimorbid patients [1,2]. Several programs, e.g. Apo-AMTS were developed to teach and implement intermediate MRs type 2a in community pharmacies according to the German MR-guideline [3,4]. But MRs are not well established in community pharmacies. 65% of all trained pharmacies conduct less than one MR in a month.
Objectives: A study protocol is developed to assess how medication reviews are successfully offered to patients in community pharmacies on a regular basis and to determine measures to foster MR implementation as a cognitive service in trained community pharmacies.
Methods: The positive deviance (PD) approach was chosen to determine favourable factors for MR implementation in community pharmacies [5]. A set of pharmacies that conducted one or more MRs in a month was generated. The different pharmacies were identified through a previous project. Eight pharmacies were selected through purposive sampling. To ensure that the identified measures are unique and adjuvant a control group (CG) of also eight pharmacies accomplishing no MRs was selected. Within each pharmacy semi-structured telephone-interviews were performed with pharmacists performing MRs (trained pharmacists), technicians and pharmacy owners/affiliation-managers. Pre-tested interview guides were developed for the use in each group. The interviews were recorded and transcripted using f4-transcript® and will be analysed following a qualitative content analysis approach with MAXQDA® 12. Additional quantitative data will be elevated to find correlations with the pharmacies` size and location, number of "medication-managers", gender, age and years of professional experience. Statistical analysis will be performed with EXCEL® 2016/SPSS® 24.
Results: All selected pharmacies were evenly distributed over Westphalia-Lippe. 44 (24 PD group/20 control group) interviews were conducted when data saturation was reached. 50% percent of pharmacy owners/affiliation managers in the PD group were male, trained pharmacists and technicians in this group were all female. In the GC group 70% of owners/affiliation managers were male as well as 12.5% in the group of the trained pharmacists all other participants in this group were female respectively. In the PD group in each partition eight participants were interviewed. In the GC with seven technicians and owners/affiliation-managers respectively and eight trained pharmacists interviews were performed. The median duration of the interviews was for trained pharmacists 19:18 [11:37-24:26] min (PD) / 11:58 [5:30-16:52] min (CG), for pharmacy owners/affiliation-managers 17:52 [10:28-26:39] min (PD) / 10:22 [6:37-19:09] min (CG) and for technicians 10:49 [5:44-17:21] min (PD) / 7:29 [5:30-13:04] min (CG). Final results from content analysis are pending.
Discussion: A trend can be seen in gender aspects. Slightly more women seem to be successful in implementing MRs into the daily routine but the higher percentage of women in Pharmacy must be considered. The complete analysis of the interviews will provide an explanation for this aspect. The longer duration of the interviews is indicative for more

information yielded from well performing pharmacies in the area of MRs. The difference is more evident for pharmacy owners showing that perhaps economical aspects need more consideration.
Conclusions: Comprehensive data-analysis with consideration of economical and gender aspects will serve to define a first set of factors for a successful, broad and sustained MR implementation type 2a in German community pharmacies. Further development of a frame-work for implementation and inclusion of patients, practitioners and stake-holders needs to follow this study.

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POS.129

Health care research in phytomedicine: PhytoVIS, a NIS in 20,870 users of herbal medicinal products

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Information on the use of herbal medicinal products is at present available only from product-specific studies, sales statistics and pharmacovigilance data [1]. The database PhytoVIS was therefore created as a tool to retrospectively document users experience with these products, in compliance to the ENCePP Code of Conduct [2].
The survey was conducted in pharmacies and doctors' practices in Germany by students of pharmacy or medicine. The only inclusion criterium was the use of herbal medicinal products within the last 8 weeks before the survey. Based on the therapeutic indication, the efficacy and tolerability of the products were endpoints. Furthermore, information on the point of sale and the recommendation of the use of the product was requested.
20,870 patients were included and their experience was documented in 24,056 questionnaires. In 78% of the participants, treatment was conducted for acute complaints, with 47% suffering from cold, flu and fever, 11% from bowel pain and digestive complaints and 9% from stomach and biliary complaints. Overall, 1,433 different products were used. In 45% of the participants, the effect of the products was described as very good and in 39% as moderate. Products were mainly recommended by the pharmacists (35%) and physicians (27%). The products were bought in the pharmacy in 84% of the cases.
PhytoVIS turned out to be a suitable tool to study tolerability, safety and efficacy based on the rating of the patients. By the high number of users included, special patient groups as toddlers, pregnant women and elderly were also covered. The database is an excellent source for medical information on the full range of herbal medicinal products in use.

Acknowledgment

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POS.130

Safety assessment of herbal products: Potential shortcomings

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Introduction: The interest in safety assessments of herbal products is increasing, but not their quality, as limitations of available data are often not sufficiently taken into account. To address the issue, a classification of sources of bias and ways out is aimed to.
Materials and Methods: A systematic data base search for reviews in this field, combined with hand searching in text books, was conducted. Sources of bias were classified according to data types involved.
Results and Discussion: Depending from the data involved, different sources of bias were identified:
Data on quality: Often the great differences of the composition of herbal products prepared from the same plant are neglected, so leading to flaws when tranferring data.
Non-clinical data: Common pitfalls are the transfer of data from in vitro studies to the clinical setting, without taking into account the influence of ADME. Often also effects from sublethal high-dose settings are used without sufficiently taking into account dose dependency or, especially e.g. in carcinogenicity studies, methodological ambiguities [1].
Data from clinical studies and post marketing surveillance: Referring to studies conducted with products of a different composition can lead to misleading attribution of efficacy to inefficient products and vice versa, lack of differentiation between negative studies and failed studies can lead to wrong conclusions on inefficacy, the evaluation of safety data is often flawed by neglecting background incidences, as e.g. in case of hepatotoxicity [2] by protopathic bias, and by the awareness and views of authors of case reports [3].
Conclusions: A higher awareness of common pitfalls in the assessment of safety data on herbal products is needed, e.g. in case of hepatotoxic risks, if we want to avoid that methodological artefacts and misperceptions of the generalizability of data continue to influence our view of the safety of herbal products, both by neglecting risks, as, more abundant, by exaggerating non-existing risks.

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POS.131

STW 5 is effective in multiple symptoms

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Introduction: STW 5 is indicated in functional dyspepsia (FD) and irritable bowel syndrome with proven efficacy and safety in several randomized controlled clinical trials (RCTs) and more than 5 decades of clinical experience [1]. However, its efficacy on single symptoms was not clinically investigated until now.
Aim of the Study: The current study closes this gap, as the available RCTs were specifically analyzed to investigate the effects on individual FD symptoms.

Materials and Methods: Of the 25 studies screened for eligibility, 6 RTCs were included, all based on the same validated symptom sum score (GIS) and 28 days of treatment duration.
Results and Discussion: Overall 637 patients were treated (STW 5: 351, placebo: 286). Mean baseline GIS sum score was 11.8 (±4.1 SD, range: 1 to 27). Overall there were no structural differences between treatment groups with respect to baseline characteristics and length of treatment.
The mean improvement of GIS sum score from baseline observed after 28 days of treatment was 7.1 points for STW 5 and 4.9 points for placebo, with a statistically significant difference of 2.07 points between the treatments regarding improvement of GIS sum score in favour of STW 5 (95%-CI: 1.37, 2.76; p<0.0001). Furthermore, the improvement of the mean score for the single items comprising the GIS was consistently and significantly in favour of STW 5 for every single item.
Conclusions: In conclusion, the analysis confirms the clinically proven efficacy of STW 5 and shows that the preparation significantly improves every single FD symptom assessed in the GIS score and ascertains its suitability for all subtypes of FD.

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POS.132

Using physiologically-based pharmacokinetic modelling to investigate the distribution of drugs into different lung tissues in rats.

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Background
The lung is an organ with a complex structure and multiple lung-specific physiological processes, which have to be considered to comprehend the pharmacokinetics (PK) of pulmonary administered drugs. The structure of the lung can be divided into the conducting airways, consisting of trachea, bronchi and the bronchioles, and the alveolar parenchyma. Apart from the different composition of these tissues [1], the conducting airways and the alveolar parenchyma are perfused differently since the conducting airways are supplied by the systemic circulation, while the alveolar parenchyma is supplied by the pulmonary circulation [2].
This study aimed (i) to investigate potential differences between the conducting airways and the alveolar parenchyma regarding the tissue-to-plasma partitioning and (ii) to evaluate estimation of lung perfusion based on two tool compounds (Fluticasone propionate (FP) and a phosphoinositide 3-kinase inhibitor (PI3Ki)).

Methods
Two studies provided in vivo PK data for both compounds in rats after an intravenous bolus (IV bolus) administration and after an infusion over one hour. Measurements were taken at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 h in the IV bolus study and at 1, 2, 3 h in the infusion study (both studies: n = 3 for each time point). In addition to plasma drug concentrations, which were measured in both studies, tissue concentrations in trachea, bronchi (upper bronchial tree to the 3rd generation) and alveolar parenchyma were measured in the infusion study. Empirical PK models were used to describe observed plasma concentration-time profiles of the tool compounds in rats after IV dosing, which were linked to physiologically-based compartments to include the measurements in the different lung tissues (trachea, bronchi, alveolar parenchyma). With the resulting PK model, tissue-to-plasma partition coefficients (Kp) were estimated for the different lung tissues and statistically compared. A stochastic simulation and estimation (SSE) approach was used to evaluate if an estimation of the blood flow to the trachea ($Q_{Trachea}$) was possible with the existing experimental data and to potentially elaborate a new study design with additional compounds and adjusted sampling times supporting estimation of $Q_{Trachea}$. SSE results were analysed regarding precision and accuracy of the estimated model parameters.

Results
The statistical comparison indicated no significant differences in tissue distribution for both compounds between trachea and bronchi, as both tissues are part of the conducting airways and have similar tissue composition. However, for both drugs a significant difference was observed between trachea (Kp of FP: 8.33, Kp of PI3Ki: 7.16) and alveolar parenchyma (Kp of FP: 11.41, Kp of PI3Ki: 13.75).
The SSE studies indicated that the tissue partitioning was estimated adequately, however, an accurate estimation of lung tissue perfusion was not possible with the existing data. Additional measurement times for tissue samples (0.25, 0.75, 2, 4 h) were identified that could allow for the estimation of $Q_{Trachea}$ without any loss in the precision of Kp estimates.

Conclusions
While the trachea and the bronchi sample were comparable in terms of tissue binding for the two model compounds after intravenous dosing, the alveolar parenchyma displayed significant differences compared to the trachea. An estimation of $Q_{Trachea}$ was not possible with the current study design, but a new study with additional compounds and adjusted sampling times will likely support estimation of $Q_{Trachea}$ and help to gain a better mechanistic understanding of the PK of inhaled drugs.

Acknowledgments: Boehringer Ingelheim Pharma GmbH & Co. KG provided funding and experimental data

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POS.133

Therapeutic usefulness of STW 5 in Irritable Bowel Syndrome (IBS) and other functional GI diseases: Results of a surveillance study in pharmacies

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Pharmacy-based surveys on therapeutic use and user satisfaction are tools to gain a better knowledge and understanding of patient needs for over-the-counter (OTC) medicinal products [1].
The herbal gastro-intestinal product STW 5 is available in the German market since 58 years [2]. A multitude of clinical, pharmacological and toxicological studies are evidence of its efficacy, safety and mode of action in the treatment of functional and motility-related gastro-intestinal diseases such as irritable bowel syndrome and functional dyspepsia [3].
Up to now, availability of consumer data on self-medication and user satisfaction of STW 5 is limited. Therefore, patients entering pharmacies with product desire or recommendation for STW 5 were asked whether they would participate in a survey on the product. To consenting customers, a questionnaire on demographic data, tolerability and satisfaction with the product was handed out, and they were asked to answer it during the next few days and send it back to a contract research institute after a week.
Overall, 844 customers of 139 pharmacies all over Germany were recruited. 29.4 % were male and 70.6 % female. The majority was in the 30-49 age range. In 384 patients, complaints were related to the upper, in 139 to the lower abdomen, in 311 patients to both regions. In 7.3 % of the 384 patients a functional dyspepsia had been diagnosed, in 16.9 % of the 139 patients an irritable bowel syndrome. The tolerability was rated good to very good in 97.4 % of all patients and did not differ between patient groups. Correspondingly 93.2 % of the users were „very satisfied“ or „satisfied“. 91 % of the customers would recommend STW 5 to others for treating their complaints.
Overall, it can be concluded that this pharmacy based survey gives a reliable picture of the behavior of users of STW 5, with high satisfaction values as well in irritable stomach as in irritable bowel syndrome. The favorable tolerability ratings confirm the ratings from the clinical studies on the product.

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3.9 Natural Compounds

POS.134

A natural biosurfactant from corn - evaluation of properties and production of nanoemulsions

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Introduction: Nowadays the use of natural detergents has been increased in different fields, such as the cosmetic and cosmeceutical industry. One big advantage is that more natural and biocompatible formulations can be obtained. Recently, a biosurfactant extract has been discovered, which was obtained in an agroindustrial residue from the corn wet-milling industry. It was shown to possesses amphiphilic and anti-oxidative characteristics that are interesting in order to substitute synthetic surfactants, providing more biocompatible formulations with added value [1]. The aim of this study was to evaluate its efficacy as emulsifier in comparison to classical and well established pharmaceutical surfactants.
Materials and methods: The biosurfactant studied in this work was extracted from corn steep liquor (BS), using the protocol established by Vecino et al. [2]. The BS was prepared by diluting the extract in water, up to a concentration of 5 g/L. In the first step, contact angle and required HLB (rHLB) were evaluated for the BS extract. And compared to commonly used surfactants such as polysorbate 80 (Tween 80), alkyl polyglycoside (Plantacare 2000), sodium lauryl sulphate (SDS) and sorbitan monolaurate (Span 20). For the investigation of the rHLB value, different formulations were prepared by using mineral oil, castor oil and peanut oil and their stability was observed during 24h. In the second step, nanoemulsions and lipid nanoparticles were produced from different lipids by high pressure homogenization [3]. These formulations were characterized in terms of size, size distribution, zeta potential and short term physical stability. Moreover, a formulation for lipid nanocarriers was produced and the co-emulsifier with similar HLB (Span 20) was substituted.
Results and Discussion: The BS possesses a good wetting ability and an HLB value around 10. The emulsions prepared by high pressure homogenization with different oils showed that the most stable emulsion (smallest size and size distribution) were obtained with mineral oil. It is necessary to remark that the ratio oil/surfactant was quite high (above 20) showing an efficient emulsion in terms of stability, especially when taking into account the low amount of biosurfactant present in the sample. Also the lipid nanocarriers, which were produced with similar production parameters as the original formulation and by only replacing the co-emulsifier Span 20 by BS, possessed a similar small size and a narrow size distribution as the original formulation [3]. The particle size was < 80nm (PdI < 0.15) and no changes in size were observed during storage at room temperature.
Conclusion: The biosurfactant extract obtained from corn demonstrated to be a suitable ingredient and stabilizer for more natural and biocompatible emulsions and lipid nanocarriers in the cosmetic and pharmaceutical fields. The big advantages of the BS are its natural origin and its additional beneficial properties, i.e. anti-oxidative properties.

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POS.135

Turning food waste into superfood and potent drug products

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Introduction: Plants' wastes, like peels and grounds of the coffees or teas, were mostly ignored, but recent studies have confirmed that nanomilling can be beneficial to turn those wastes into value [1]. Since Acorn nut and its coffee have good antioxidant capacities due to the high tannis content [2], their remainings were chosen to be tested for their antioxidant activity.
Aim: The aim of this study was to investigate the antioxidant activity of the nanosuspensions of the wastes of Acorn nut and Acorn coffee.
Materials & methods: The Acorn nuts were collected in Saarbrücken and separated into two parts: the inner seeds and the peels (waste). Acorn coffee was purchased in a roasted form from a Reformhaus then boiled to obtain its ground (waste). Both samples were dried and grinded to fine powder, then suspended in a surfactant solution of Tween 80®. High Speed Stirring (HSS) was applied on the obtained bulk-suspension to form a micro-suspension. Furthermore, the resulted suspension was homogenized by using High Pressure Homoginization (HPH). During and after the milling process, particles sizes were analyzed using Photon correlation Spectroscopy (PCS), Laser Diffraction (LD), and Light Microscopy [2]. The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed to detect the antioxidant activity of the produced wastes' nanosuspensions and compared to those activities of the plants' nanosuspensions, where ascorbic acid was used as standard (lowest IC50).
Results and discussion: The results show that it is possible to reuse plants remainings and convert them to valuable active products through nanosizing. The combination of HSS and HPH techniques provided nanosuspensions of the plant materials and their wastes with particle sizes in the range of 400-700 nm. The obtained nanosuspensions showed good antioxidant capacity (Fig.1). In its nano form, the peel of the acorn nut showed a higher antioxidant activity with an IC50 of 0.05 mg/ml compared to the inner seed which was less active showing an IC50 of 0.2 mg/ml. Likewise, coffee waste nanosuspensions showed a higher antioxidant activity with IC50 of 0.02 mg/ml compared to the original raw roasted coffee which has slightly lower antioxidant activity with IC50 of 0.03 mg/ml.

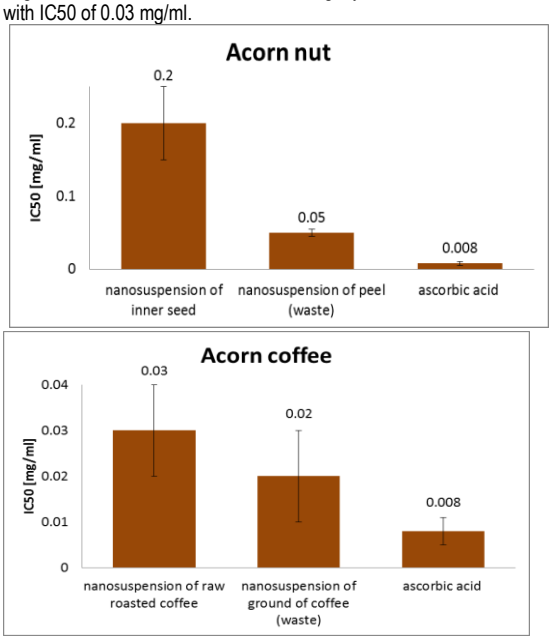

Fig.(1): IC50 of Acorn nut and its waste nanosuspensions and Acorn coffee and its waste nano-suspensions.

Conclusion: By nanomilling, we could obtain nanosuspensions from the waste of the mentioned plant materials with similar or even higher antioxidant capacity in comparison to the original plant nanosuspensions.

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POS.136

Isolation and identification of five flavonoid glycosides from *Ornithopus compressus* L. including three formerly undescribed natural products

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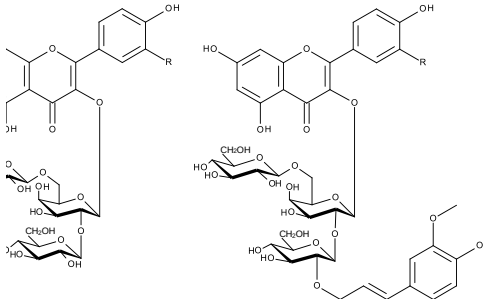
Ornithopus compressus L., yellow serradella (Fabaceae), is an annual pasture legume natively occurring in the Mediterranean, Western Asia, the Caucasus area, and Macaronesia [1]. The species is cultivated and used in livestock forage production and as a pasture legume mainly in Australia and Chile [2,3]. Secondary metabolites detected in the leaves thus far comprise the biogenic volatile organic compounds (BVOCs), monoterpenes α-pinene, β-pinene, (+)-4-carene, camphene, camphor, limonene, and isoborneol [4] as well as isoflavones daidzin, genistin, daidzein, glycitein, genistein, pratensein, formononetin, irilone, prunetin, and biochanin A [5,6]. We isolated and analyzed five flavonoid glycosides from *Ornithopus compressus* leaves from Pozzuoli near Naples, Italy. Structures of the isolated compounds were elucidated as kaempferol 3-*O*-(2,6-di-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**1**), kaempferol 3-*O*-(2,6-di-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**2**), quercetin 3-*O*-(2,6-di-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**3**), quercetin 3-*O*-β-D-glucopyranosyl-(1→6)-[2-*O*-(2E)-feruloyl-β-glucopyranosyl]-(1→2)-β-D-galactopyranoside (**4**), and isorhamnetin 3-*O*-(2,6-di-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**5**). All five compounds are described from *Ornithopus compressus* for the first time. Compounds **1** and **3** have been described from *Tofieldia nuda* Maxim. (Tofieldiaceae) [7], while compounds **2**, **4**, and **5** represent new natural products. For structure elucidation HPLC-DAD, HPLC-MS, and NMR (^{1}H NMR, ^{13}C NMR, H,H-COSY, HMBC, HSQC) were applied.

Isolated compounds:



1: R = H, **3**: R = OH, **5**: R = OCH_3 **2**: R = H, **4**: R = OH

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POS.137

PST.06

Investigations into the influence of pretubulysin (PT) and other microtubule-targeting agents (MTAs) on leukocyte-endothelial cell interactions

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The tightly regulated process of inflammation is strongly required for providing protection against infections or injury in the healthy organism [1]. However, non-resolving inflammation and inflammatory disorders play a crucial role in a broad variety of diseases like autoimmune diseases, characterized by constant infiltration of leukocytes leading to tissue damage and even cancer [2]. In this regard, microtubule-targeting agents (MTAs), primarily known for their use as chemotherapeutic drugs, have often been described as anti-inflammatory substances with colchicine as a lead compound [3, 4]. The aim of this study is therefore to investigate the impact of the novel MTA pretubulysin (PT) and of already established MTAs on leukocyte-endothelial cell interactions to proof the anti-inflammatory potential of these drugs. Since the role of the endothelium has been largely neglected in the development of anti-inflammatory compounds, this work mainly focuses on the influence of MTAs on inflammatory processes of endothelial cells.

Intravital microscopy of postcapillary venules of the mouse cremaster muscle revealed that the treatment of mice with PT decreases the TNFα-induced firm adhesion of leukocytes onto and their transmigration through the vascular endothelium *in vivo*, whereas the rolling of leukocytes on the vascular surface was not affected by the compound. Moreover, *in vitro* cell adhesion assays showed that the adhesion of monocytic cells (THP-1) onto TNFα-activated endothelial cells is reduced when endothelial cells were pre-treated with the microtubule-destabilizing drugs PT, vincristine (VIN) or colchicine (COL). In contrast, the TNFα-induced adhesion of THP-1 cells onto an endothelial cell monolayer was not impaired when endothelial cells were pre-treated with the microtubule-stabilizing agent paclitaxel (PAC). It could be further shown that the treatment of primary human monocytes with PT reduces the adhesion of monocytes onto TNFα-activated endothelial cells in *in vitro* cell adhesion assays. Based on this data, the influence of PT and other MTAs on the surface expression of the cell adhesion molecules (CAMs) ICAM-1 and VCAM-1 (on endothelial cells) and of the CAMs LFA-1 and VLA-4 (on primary human monocytes) were determined by flow cytometry. While the TNFα-induced surface expression of ICAM-1 and VCAM-1 is decreased by the pre-treatment of endothelial cells with PT, VIN and COL, the surface expression of ICAM-1 was not and that of VCAM-1 only slightly affected when TNFα-activated endothelial cells were pre-treated with PAC. Moreover, it could be demonstrated that the surface expression of both LFA-1 and VLA-4 is reduced in PT-treated primary human monocytes. It cannot be excluded that this reduction is caused by effects of the used MTAs on the microtubule-mediated transport of the examined CAMs. Nonetheless, western blot and qRT-PCR analysis revealed that the total protein expression and the gene expression of both ICAM-1 and VCAM-1 are down-regulated by PT, VIN and COL in TNFα-activated endothelial cells. In line with the data revealed by flow cytometry, the TNFα-induced total protein expression and gene expression of ICAM-1 was not and that of VCAM-1 only slightly affected by PAC treatment. Based on these findings, the influence of PT and other MTAs on the NFκB promotor activity was determined by a dual luciferase reporter assay. It could be shown that the pre-treatment of TNFα-activated endothelial cells with PT, VIN and COL causes a reduction of the promotor activity, whereas this process was not affected when endothelial cells were treated with PAC.

Taken together this study shows that the microtubule-destabilizing agents PT, VIN and COL interfere with leukocyte-endothelial cells interactions. At least in part, this influence is caused by an effect of these MTAs on the NFκB signaling cascade in endothelial cells, whereas further studies are required to investigate the influence of MTAs on NFκB upstream targets and also other interacting signaling pathways.

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POS.138

Click & shine – Fluorescence labelling of microcystins

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The recent development of bioorthogonal crosslinking techniques – so-called "click reactions"– offers new possibilities to study the function of compounds in cellular processes [1]. They allow for an easy conjugation of the molecules of interest with probes and, thus, make them visible by suitable imaging techniques. However, the biorthogonal reactions require functional groups not found in nature, so they need to be introduced synthetically, which can be a challenging task for natural products.
Our molecules of interest – the cyclic heptapeptides microcystins – belong to the best-studied natural products from cyanobacteria, but their physiological role within the host remains unclear [2]. Some findings suggest that they play a crucial role in the adaptation of the cyanobacteria to high light conditions [3]. However, still little is known about the underlying mechanisms, the localization of microcystins in the producing cell, or their interaction partners.
Making microcystins amenable for bioorthogonal chemistry might result in new insights into their biological function and the physiology of cyanobacteria. After labelling with a probe, the microcystins can be localized by microscopic means, or their interaction partners can be identified by proteomics-based approaches. Thus, we present a rapid and feasible technique to introduce azide- and alkyne groups into microcystins: precursor directed biosynthesis. Azide and alkyne functionalized tyrosine derivatives were added to a microcystin-YR producing Microcystis sp. strain and successfully incorporated into this congener. This allowed for the conjugation of the "clickable" microcystin with e.g. a fluorescent dye and shed a new light on their role in our subsequent physiological studies, of which first results are presented.

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POS.139

Natural products and the liver: Hepatoprotection as a key

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Introduction: While in Europe the scientific awareness in the field of liver and natural products is presently mainly focused on herb induced liver injury (HILI) [1], in Asia, the focus of a number of recently published reviews is on hepatoprotective effects of herbs [2]. But also in Europe, there is a high prevalence of hepatic disturbances due to different causes, so that an overview of the hepatoprotective potential of herbs in use in Europe would be of therapeutic relevance, e.g. in herbs used in the therapy of gastrointestinal diseases.
Materials and Methods: For identifying these plants, the HMPC monographs of the European drug agency EMA and in addition leading textbooks were evaluated, followed by a systematic literature search on hepatoprotective effects.
Results and Discussion: There are HMPC monographs describing a use in gastrointestinal diseases for 50 medicinal herbs, others are described in textbooks. For the majority of these plants there are published data on hepatoprotective effects. In most cases these are data from pharmacological models of different types of liver diseases, as are e.g. for caraway, peppermint, lemon balm, liquorice, gentian, angelica root, but also fennel and greater celandine. In specific cases there exist also data from clinical trials, as e.g. case of artichoke and milk thistle. In addition there are data on herbal constituents as e.g. quercetin und sulforaphane available, which point to hepatoprotective effects of further plants, as e.g. bitter candytuft or horseradish.
Conclusions: The herbs used in Europe have in many cases a hepatoprotective action, as e.g. in gastrointestinal diseases. By means of pharmacoepidemiological studies it could be evaluated whether this action is reflected in the respective patients also in a lower incidence of hepatitis of different origin.

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3.10 Pharmaceutical Technology and Biomaterials

POS.140

Development of a green preparation method for atorvastatin and rosuvastatin nanoparticles for the treatment of inflammatory diseases

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Statins are highly potent suppressors of the hepatic cholesterol biosynthesis through inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and therefore widely used as cholesterol lowering drugs. In addition, statins show pleiotropic effects such as anti-inflammatory properties and immunomodulation and offer a potential to treat inflammatory diseases. [1] Atorvastatin calcium (AC) and rosuvastatin calcium (RC) are BCS class II drugs which suffer from poor water solubility and systemic bioavailability [2,3]. Therefore, biodegradable nanoparticular drug delivery systems are a promising approach to overcome these poor physicochemical properties and to enhance the bioavailability. The aim of this study was the development of poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NP) with a green organic solvent-free preparation method [4] for the lipophilic statin AC and hydrophilic statin RC as biocompatible and active drug delivery systems for the treatment of inflammatory diseases.
Blank particles and particles loaded with AC and RC were prepared by a green organic solvent-free method and an emulsion evaporation method [5] using different types of PLGA. The physicochemical properties of the NPs were characterized for their hydrodynamic diameter, polydispersity index, zeta potential and morphology by photon correlation spectroscopy, laser Doppler anemometry and scanning electron microscopy (SEM), respectively. Furthermore, the drug load (DL) of the NPs was determined by HPLC with UV/VIS detection and the *in vitro* drug release was investigated for up to 7 d in phosphate-buffered saline (PBS) pH 7.4. Additionally, the NP were tested for their *in vitro* hemocompatibility using isolated sheep erythrocytes (RBC) as well as their *ex ovo* biocompatibility, using a more complex shell less hen's egg model (HET-CAV).
Nanoparticles prepared by emulsion evaporation method showed a hydrodynamic diameter between 180 nm and 250 nm for AC-NP and RC-NP, with a narrow particle size distribution (PDI < 0.2). The zeta potential indicated a stable nanoparticle dispersion and was in the range between – 21 and – 32 mV. The blank particles showed comparable particle size and zeta potential, indicating that the drug load had no influence on these parameters. The particles prepared with the green organic solvent free method showed comparable hydrodynamic diameter and zeta potential, as well as a narrow particles size distribution. The DL was higher for AC-NP as for RC-NP using this method. The *in vitro* drug release rate of the AC-NP and RC-NP was found to be dependent on the molar mass of the PLGA polymer and decreased with higher molar masses. Furthermore, the AC-NP and RC-NP showed no *in vitro* hemotoxicity after incubation with isolated RBCs and a good biocompatibility after systemic injection in the dynamic blood flow of the hen's egg test. In ongoing experiments, AC-NP and RC-NP are assessed for anti-inflammatory properties (cytokine release) in lipopolysaccharide-stimulated human monocytes.
In conclusion, the green method is a preparation technique applicable for both statins and results in comparable particle properties in comparison to the emulsion evaporation method. A toxic and environmental harmful organic solvent can be avoided successful with the green preparation method.

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POS.141

Hyperforin stabilization by nanoemulsions processed in microsystems

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Hyperforin is an active pharmaceutical ingredient from *Hypericum perforatum* with promising therapeutical options [1]. However, a major challenge is its low chemical stability. Due to the insufficient chemical stability of the natural hyperforin molecule, a stable carrier system such as nanoemulsions is required. Nanoemulsions join various advantages from nanodisperse formulations as for example improved drug release and higher drug absorption. Therefore, they are widely examined as carriers for drugs with poor water solubility. Previous studies have shown that albumin stabilizes the hyperforin dicyclohexylammonium salt (HYP-DCHA) [2]. Therefore, the influence of albumin addition into the outer phase of nanoemulsions was also evaluated with regard to HYP-DCHA stability.

Nanoemulsions were prepared using a novel microchannel high pressure homogenization device [3, 4] recently developed in our group. The oil phase of the formulation consists of medium-chain triglycerides (Miglyol® 812, Caesar & Loretz GmbH) with various concentrations of hyperforin dicyclohexylammonium salt (HYP-DCHA, Phytolab). Together with a solution of 3 % macrogol-15-hydroxystearate (Solutol® HS15, BASF) in double distilled water (all w/w) [3], 5 % of the oil phase was pre-emulsified with a rotor-stator high shear mixer (Ultra-Turrax®, IKA, Staufen) equipped with S25 N-10G dispersion tool at 12.000 1/min for 20 s. This pre-emulsion was cycled through the microchannel geometry under constant conditions.

A setup with a homogenization pressure of 1240 bar and 3 cycles rendered a narrow particle size distribution with a d_{10} value of 64 nm, a d_{50} value of 96 nm and a d_{90} value of 148 nm, which are stable over two months.
The recovery of HYP-DCHA was quantified by reversed-phase HPLC technique. Compared to other drug delivery systems such as solid lipid nanoparticles, the recovery of HYP-DCHA was significantly higher (49% ± 10 %). Adding BSA to the aqueous phase slowed down the degradation of HYP-DCHA, which led to a HYP-DCHA recovery of 86 % ± 14 % (n=3). These HYP-DCHA concentrations were stable over a month.
Furthermore, the effect of loaded nanoemulsions on the viability of a human keratinocyte cell line (HaCaT cells) was examined with an MTT-assay. Higher HYP-DCHA concentrations, especially in samples with additional BSA, decreased the viability. This also confirms the stabilization of HYP-DCHA through the combination of nanoemulsions and BSA.

Acknowledgments:

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POS.142

Application of an external phase separation technique to investigate drug loading of lipid nanoemulsion droplets

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An increasing number of poorly water-soluble drugs in the development pipeline require strategies for their formulation. A possible strategy can be formulation by incorporating them into lipid nanoemulsions. In nanoemulsions, drugs can be localized in or at the surface of lipid droplets as well as in the aqueous phase, for example in further colloidal structures formed by an excess of emulsifier (e.g., micelles). Due to the very small particle sizes, localization at interfaces, whose properties are mainly determined by the emulsifier, may play a major role. Thus, it is important to investigate effects on drug loading caused by an exchange of emulsifier.

In order to be able to evaluate the absolute drug content in lipid carrier particles, it is important to determine the amount of drug in the aqueous phase. Knowledge about the precise composition of the external aqueous phase is a prerequisite to determine solubilities in it. The amount of surfactant required to stabilize an emulsion of given particle size depends on the type of emulsifier. Therefore, it can be expected that the concentration of emulsifier remaining in the aqueous phase differs if different emulsifiers are used for emulsion preparation.

A method to separate the aqueous phase from the lipid particles in order to determine its emulsifier content was developed and validated. In the literature, ultrafiltration or centrifugation are described to separate the external aqueous phase from nanocarriers [1,2]. In the present study, the applicability of a Vivaspin® centrifugation device-based method to determine the composition of the external aqueous phase in emulsions was investigated. Vivaspin® tubes with a PES membrane of 300.000 Da MWCO were used with a moderate centrifugation speed of 500 g. In the separated external phase, the amount of unbound emulsifier was determined by refractometry. The method was validated in terms of emulsion stability during centrifugation, emulsifier passage and possible emulsifier retention in the supernatant. Furthermore, drug adsorption to the ultrafiltration membrane was investigated to explore the possibility of direct determination of drug content in the separated external phase of drug-loaded nanoemulsions.

After validation, the Vivaspin® technique was used to investigate the effect of emulsifier exchange on the achievable drug load in lipid nanoemulsions. Four different trimyristin nanoemulsions (5 %) were prepared with sodium azide as preservative. The emulsifier was different in each emulsion: poloxamer 188, poloxamer 407, polysorbate 80 or tyloxapol. All emulsions had a similar mean droplet size of 109 ± 6 nm. For all emulsions, the aqueous phase was separated and the emulsifier content was determined. Emulsions were loaded with betamethasone valerate or fenofibrate, using a passive drug loading protocol. In this procedure, the preformed drug-free nanoemulsion was added to an excess of drug [1]. After an adequate incubation time dissolved drug was removed by filtration. To determine the drug solubility in the aqueous phases, emulsifier solutions with comparable emulsifier contents were prepared. These emulsifier solutions were saturated with drugs, applying the same passive loading procedure as used for drug loading into emulsions. The drug content in loaded nanoemulsions and emulsifier solutions was determined by UV spectroscopy in tetrahydrofuran/acetonitrile solvent. To obtain the absolute amount of drug loaded to the emulsion droplets, the amount of drug loading in the emulsions was corrected by subtraction of the drug content determined for the corresponding aqueous phase.

The achievable fenofibrate load did not depend on the emulsifier. For betamethasone valerate, in contrast, a significantly higher load was observed in poloxamer 188-stabilized emulsion droplets than in other emulsions. This is probably related to the fact that betamethasone valerate is preferably localized in the droplet surface, where it is stronger influenced by the emulsifier, whereas fenofibrate is preferably localized in the droplet core [3].

Drug load may thus exhibit a significant variability depending on the type of emulsifier used for emulsion stabilization, but not in all cases is drug load influenced by an emulsifier change. The loading differences observed for betamethasone valerate were only detectable when applying the phase separation technique. The drugs had different solubilities in the aqueous phases of the differently stabilized emulsions. The determination of these solubilities was thus essential to elucidate absolute drug loading of the emulsion droplets.

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POS.143

Development of a UHPLC quantification of polysorbate 80 using oleic acid derivatives

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Quantification of polysorbate 80 is challenging as it is a mixture of various substances [1,2]. Mainly it consists of sorbitol esterified with oleic acid and etherified with polyethylene glycol of varying chain lengths. Byproducts of the synthesis are additional fatty acids and the starting materials. As those influence the formation of micelles and the stability of pharmaceutical formulations [3], knowledge of the composition is crucial. Also for the determination of the content of formulation ingredients, the concentration of the emulsifier is important to know in order to estimate micellar interactions. Therefore, an analytical method for polysorbate 80 quantification in aqueous solution at low concentrations had to be developed.

Using the Design of Experiments (DoE) software Drylab®, chromatographic methods with MS-detection were developed. The UHPLC was coupled to a QDa. At first, peak separation of all fatty acids, which might occur in polysorbate 80 according to the Ph. Eur. [4], was achieved (with varying retention times and mass-to-charge ratios). Quantification using the oleic acid peak could not be achieved as a double peak was found. As it had the same mass-to-charge-ratio, an isomer of the C18:1 acid was proposed, which might be petroselinic acid according to literature [5]. Running the method with 6 possible isomers including elaidic acid, petroselinic acid, petroselaidic acid, cis- and trans-vaccenic acid using the general fatty acid method showed that a change in the method is necessary to achieve peak separation. Even with a newly calculated method a successful separation of all peaks was not achieved as an overlay of the peaks of petroselinic, elaidic and trans-vaccenic acid occurred, while a separation of the cis- and trans-isomers was possible. Therefore, the oleic acid isomers were derivatized with potassium permanganate to dihydroxystearic acid (see Figure 1). The derivatives showed good peak separation. The analysis of polysorbate 80 revealed that besides oleic acid also elaidic and cis-vaccenic were found. Trans-vaccenic acid, which is mostly formed in animal tissue [6], was not found, as the oleic acid was extracted from olive oil. Calibration was achieved in a range of 0.046-5.83 µg mL^{-1}.

Quantification of polysorbate 80 using derivatized oleic acid showed good reproducibility and linearity at low concentrations.

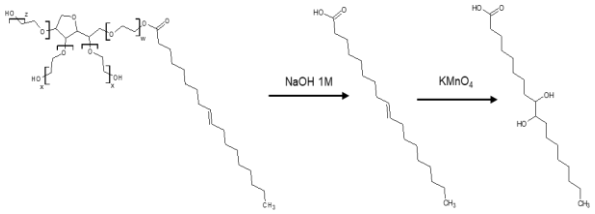

Figure 1 polysorbate 80 hydrolysis and derivatization

Acknowledgments: This work was financially supported by Almirall Hermal GmbH

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POS.144

PST.07

Electrospun Bioactive Wound Dressing containing Nanodispersions of Birch Bark Extract

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The human skin serves as a barrier between the body and the environment. Therefore, it is prone to microbial, thermal, mechanical and chemical threats which can cause acute or chronic wounds. Triterpenes from the outer bark of birch are known for various pharmacological effects including enhanced wound healing. Polymeric nanofibers made from biodegradable and biocompatible synthetic or natural polymers have been utilized to develop drug delivery systems to treat various ailments and one of the potential area to use them is medicated wound dressing. Hence, the main aim in this work is to use birch bark dry extract (TE) which has been recently clinically proved to speed up wound healing and develop a bioactive nanofiber wound dressing. Sub-micron o/w-emulsions containing TE as the active substance were prepared by high shear and high-pressure homogenization (HPH) techniques using lecithin-based phospholipids as emulsifier. These emulsions were blended in different proportions with aqueous polyvinyl alcohol (PVA) solutions and were then electrospun to form nanofibers intended for wound therapy. The influence of emulsion compositions (polymer/drug/phospholipid/sunflower oil) towards the drug release behaviour of the scaffolds together with their compatibility, surface morphology, and thermal properties were evaluated. Scanning electron microscopy (SEM) revealed that nanofibers prepared with low PVA polymer concentration resulted to non-uniform beaded fibers whereas fibers containing high concentration of PVA were smooth and uniform having an average diameter of approximately 358 nm. Confocal Raman spectral imaging proved a good encapsulation of triterpenes and compatibility of all components within the nanofiber mat. Both FT-IR and differential scanning calorimetry (DSC) analysis also illustrated that intermolecular interactions between TE and other constituents might take place during the preparation of wound dressing. In vitro drug release studies indicated that the electrospun nanofibers showed initially a significant burst release followed by a sustained release of betulin, the main component of birch bark dry extract, making the examined dressings highly applicable for several wound care applications. Therefore, our results conclude that the developed triterpene-based dressing shows promising potential for wound therapy, an area where effective remedy is needed.

Acknowledgments

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POS.145

Breaking the Biofilm Barrier: Polymer Particles for Antibiotic Applications in Biofilm-embedded Pathogens

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The antibiotic drug delivery in lung infections is very challenging: Not only the lung mucus but also bacterial biofilms limit the deposition of antibiotics due to interactions with polyanionic components and decreased diffusion rates [1]. To overcome the mucus and biofilm barrier nanoparticulate polymeric carriers are promising to increase the deposition of antibiotics to the bacteria [2]. For the preparation of nanoparticles as well as their physicochemical and biological characteristics the use of stabilizers is crucial. In this study, different polymers were screened for their capability to stabilize poly(lactic-co-glycolic acid) (PLGA) particles by utilizing a single emulsion method [3]. Selected stabilizers were chosen to prepare fluorescently labeled particles to test permeation through artificial mucus (AM) and penetration into biofilms of *Burkholderia cepacia* and *Staphylococcus aureus*.

Particles were prepared by adding PLGA solved in ethyl acetate to the aqueous phase consisting of the stabilizer in concentration of 0.01 % to 1.0 % (chitosan hydrochloride (Cs), sodium alginate) or 0.1 % to 10 % (polyvinyl alcohol (PVA), poly(vinyl pyrrolidone) (PVP), poly(ethylene glycol) (PEG), poly(2-ethyl-2-oxazoline) (POx), poloxamer and dextran). The obtained emulsions were homogenized for 15 min by Ultra-Turrax T25 (IKA-Werke, Staufen, Germany), washed by centrifugation and physicochemically characterized by measuring the hydrodynamic diameter (HD) and zeta potential (ZP). Poloxamers and PVA with concentrations of 1 % to 10 % were able to form particles with HDs below 250 nm. The use of POx (250 kDa, 1 % to 10 %) led also to particles with an HD below 250 nm but a low yield. All particles, except for Cs-particles, showed negative ZPs. In contrast, depending on concentration Cs-particles exhibited ZPs from +10 mV (0.01 %) to +40 mV (1 %).

PVA, PEG, POx, poloxamer 188 and Cs as well as covalently bound PEG-PLGA (with 0.2 % PVA) were chosen to prepare fluorescently labeled particles. The particles were characterized by scanning electron microscopy, dynamic light scattering, ZP measurement and infrared spectroscopy. All particles revealed HDs of about 600 nm to 800 nm and negative ZPs of about -30 mV, except for Cs-particles which showed a positive ZP (+10 mV). The permeation ability was analyzed in ThinCert™ cell culture inserts for 24-well plates (PET membrane, 8 µm; Greiner bio-one® GmbH, Frickenhausen, Germany) by measuring the fluorescence intensity of the particles in the lower compartment after permeating through AM (upper compartment) over time. After 24 h, all particle types permeated through AM but the largest percentages in the lower compartment were found for POx and PEG-PLGA particles.

Biofilms of *B. cepacia* (ATCC 25416) and *S. aureus* (ATCC 43300) were grown at 37 °C for at least 48 h. Particle suspensions were added to the biofilms and incubated for 4 h followed by LIVE/DEAD® staining and observation by confocal laser scanning microscopy. All particle types were visible in the biofilms but PEG-PLGA based particles were found in deeper layers indicating their excellent penetration ability. As proof of concept, the antibiotic tobramycin was encapsulated in these particles [4]. In comparison to the free drug Tb particles showed superior efficacy against biofilm-embedded *B. cepacia* as shown by the increased detection of dead cells. Blends of free Tb and drug-free particles had no antimicrobial activity indicating that only Tb loaded particles can reach the biofilm embedded bacteria.

In conclusion, different polymers were determined and identified as stabilizer of PLGA-based particles. Due to variation of the particle formulation, particle properties such as physicochemical characteristics, penetration into bacterial biofilms and AM could be modified. Moreover, it could be shown that biodegradable polymers exhibit a great potential as micro- and nanocarrier systems for antibiotics to improve their deposition and efficacy in deeper biofilm layers to provide therapeutic benefit in biofilm-associated pulmonary infections.

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POS.146

Formulating Probiotics as Mucoadhesive, Fast Disintegrating Tablet

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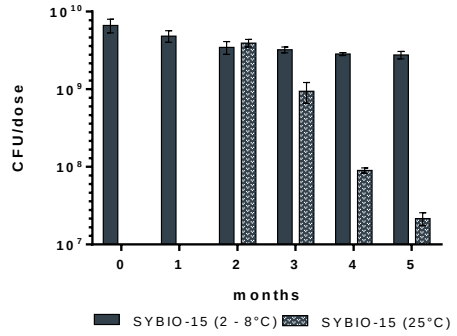
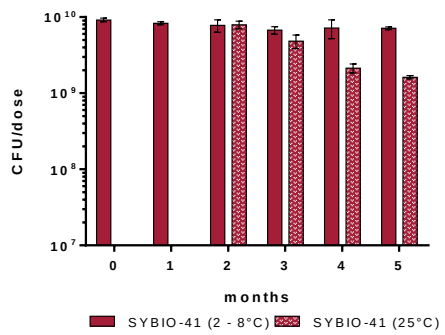
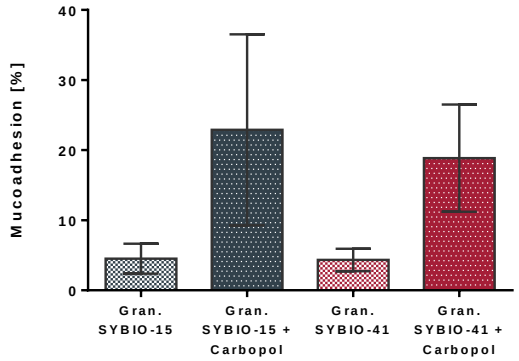
Lactobacillus species are part of the microbiome of the oral cavity and classified as probiotics. Positive action like anti-inflammatory effects [1,2] or antimicrobial activity against *P. gingivalis* [3] are described. Thus, Lactobacillus might be an option to fight inflammatory state like gingivitis. For convenient application, a suitable dosage form, e.g. orodispersible tablets (ODTs), is required. Furthermore, the released probiotics should be retained in the oral cavity which could be accomplished by the use of a mucoadhesive polymer like Carbopol 971P NF.
The probiotic material or probiotics plus Carbopol were granulated with an acrylates copolymer solution to achieve fast tablet disintegration. ODTs were produced by direct compression and were characterized by their hardness and disintegration time. Storage stability of tablets was assessed in tightly sealed packages at 2 - 8 °C and 25 °C & 60 % relative humidity. Mucoadhesion was tested in a purpose-built testing set-up where the tablets were placed on porcine buccal mucosa and were flushed with artificial saliva (0.5 ml/min) for 60 min. The percentage of mucoadhesion was calculated at the end of the experiment from the bacterial count on the mucosa and the originally applied number of bacteria.
Granulation of the probiotic powder allowed to bind Carbopol 971P NF tightly to the bacteria and guaranteed fast disintegration of the ODTs (< 30 s). Moreover, granulation was not harmful to the probiotics and the survival rate was still high after compression. The storage stability of both tested strains was high under refrigerated conditions over 5 months, whereas SYMBIO-41 was more stable during storage at 25 °C compared to SYMBIO-15.





The addition of Carbopol 971P NF allowed 20 % of the applied bacteria to stick to the mucosa, whereas without Carbopol only 5 % were retained.



The study proved that it is possible to deliver probiotic bacteria in a convenient to use, stable and mucoadhesive ODT which can be readily used to treat inflammation in the oral cavity.

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POS.147

Bacterial nanocellulose loaded with lipophilic frankincense extract as tailor-made wound dressing to treat inflamed wounds

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Age-associated inflammatory diseases such as arthrosis or Alzheimer disease are widely spread, and their treatment is intensively investigated. Frankincense is a promising natural product to treat these diseases with outstanding anti-inflammatory characteristics, which suffers from poor oral bioavailability. An innovative delivery approach to overcome this challenge is bacterial nanocellulose (BNC) as a dermal drug carrier. BNC is a biopolymer with exceptional physicochemical and biological characteristics. A unique three-dimensional network of nanosized fibers, excellent biocompatibility and high purity enables the use of BNC for medical applications such as implants, tissue scaffolds or wound dressings [1]. In our studies, BNC was loaded with different natural lipophilic molecules with high anti-inflammatory effects. Since it is still challenging to load the highly hydrophilic BNC with lipophilic substances the focus of this study was to evaluate loading efficiency and release behavior of the lipophilic frankincense extract, which is comprised of the lead substances 3-O-acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA). The influence of different loading techniques, added excipients and utilization of nanoemulsions was examined.
BNC was synthesized by the bacterial strain *Komagataeibacter xylinus* at 28 °C in either lab-scale in 24-well plates for 14 days [2] or upscaled in trays (20 x 20 cm) for seven days. Dimension, weight, compression stability, water retention and water absorption ability of the BNC fleeces were determined and compared. BNC was loaded with frankincense extract by absorption or reswelling method using poly(ethylene glycol) and Poloxamers as additional excipients or nanoemulsions. The physicochemical characteristics as well as the loading efficiency of the BNC fleeces after using different loading techniques and additional excipients were examined. The release behavior was investigated by using Franz diffusion cells at 32 °C. To determine the depth of penetration into skin, tape stripping experiments were performed utilizing

porcine skin. In addition, the nanoemulsions were characterized regarding particle size distribution, hydrodynamic diameter, zeta potential and storage stability.
BNC cultivated in 24-well plates and trays showed similar dimensions and weights. Water absorption, water retention and compression stability values of lab-scale and upscale BNC were comparable. Due to improved efficiency, the upscale process was selected to synthesize BNC for further loading and release studies. While the BNC loaded with the additional excipients poly(ethylene glycol) and Poloxamer 407 in combination with frankincense influenced the compression stability, water absorption and water retention ability, the utilization of nanoemulsions revealed no relevant changes of the compression stability. The release profiles of AKBA and KBA could be modified by using different excipients or nanoemulsions. Penetration studies showed that AKBA and KBA could be detected in deeper layers of porcine skin. Depth and amount of frankincense varied in accordance to the utilized excipient.
By varying the loading technique, added excipients and formulation strategy, we were able to effectively load BNC with lipophilic frankincense molecules and to realize controlled release profiles of AKBA and KBA to develop an innovative tailor-made dressing for chronically inflamed wounds.

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POS.148

The role of different types and arrangements of cationic charges in poly(methacrylate) copolymers for gene delivery

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Nonviral gene therapy is a steadily growing field with great clinical promise that avoids many problems associated with viral gene therapy. Polymer based vectors offer a versatile, cost-effective alternative to viral gene delivery. However, discrete structure-activity relationships (SARs) have yet to be defined in order to produce highly efficient, non-toxic delivery systems. In this study, the influence of origin and distribution of cationic charges in polymeric vectors was investigated in terms of DNA binding, polyplex formation as well as *in vitro* toxicity and gene delivery. While the highly biocompatible *N*-(2-hydroxypropyl)methacrylamide (HPMA) [1,2] served as the neutral part of the polymer, *N*-(3-aminopropyl)methacrylamide (APMA) and *N*-(3-guanidinopropyl)methacrylamide (GPMA) were chosen as cationic moieties. Together with an increase of electrostatic interactions, the guanidinium group in GPMA is able to establish strong hydrogen bonds with DNA [3,4], which makes it a promising structural feature to enhance efficient gene delivery.
Two series of each HPMA/APMA and HPMA/GPMA copolymers with increasing cationic comonomer content from 5 mol% to 90 mol% were synthesized by aqueous reversible addition-fragmentation chain transfer (aRAFT) polymerization to yield monodisperse (Đ ~ 1.2) polymers that are cationic at physiological pH. The two series differed in the distribution of the cationic comonomer, which is either gradient or statistically distributed throughout the polymer chain. Plasmid DNA (pDNA) binding was quantified by fluorophore exclusion assay. Protection from enzymatic degradation was evaluated by horizontal agarose gel electrophoresis. The hydrodynamic diameters and zeta potentials were determined by dynamic light scattering. *In vitro* cytotoxicity was performed by cellular MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction. Transfection efficiency was tested
48 h after treatment with polyplexes by measuring transgene luciferase activity in CHO-K1 cells.
Gradient copolymers exhibited a higher pDNA binding efficiency in comparison to statistic counterparts for both, APMA and GPMA derivatives. GPMA outperformed APMA counterparts in terms of pDNA affinity, already exhibiting quantitative binding at only 5 mol% GPMA content. At comonomer contents of 40 mol% or higher, all polymers protected the pDNA payload from enzymatic degradation and formed nanoscaled polyplexes (~100 nm) of high cationic charge (30-50 mV) that increased with the comonomer content. Half-maximal inhibitory concentrations (IC_{50}) demonstrated a dose- and comonomer content-dependent toxicity of the polymers. While gradient counterparts displayed a higher toxicity, GPMA-containing polymers were less toxic than APMA derivatives. Additionally, GPMA copolymers exhibited efficient transfection at 40 mol% comonomer content which was only achieved at 90 mol% for APMA derivatives. Statistical copolymers showed a higher transfection efficiency than gradient counterparts and GPMA copolymers induced a higher transgene expression than APMA derivatives. The most pronounced transgene expression was achieved at 90 mol% comonomer content for both, APMA and GPMA copolymers.
In conclusion, the type of cationic charge and its arrangement in copolymers play a critical role in the functionality of nonviral vectors for nucleic acid delivery. Considering the demonstrated SARs may help to optimize polymeric vectors and to overcome current limitations of nonviral gene delivery.

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POS.149

Post- and *in situ-*modification approaches for bacterial cellulose wound dressings to facilitate lipophilic natural drug incorporation

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Bacterial cellulose (BC) is a biotechnologically derived nanostructured biopolymer with high potential in applications such as wound management or drug delivery systems [e.g. 1, 2]. It combines typical cellulose properties such as an excellent biocompatibility with the features of nanostructured materials, among them a high mechanical stability as well as a high liquid loading and holding capacity.
In the InflammAging project, anti-inflammatory natural substances, such as Vitamin E metabolites and triterpene acids from frankincense [3], are investigated as innovative active pharmaceutical ingredients. The most promising candidates are incorporated in BC-based wound dressings in order to treat local inflammatory wounds. Compared to a systemic administration, such a dermal application would increase the bioavailability and safety, good tolerance and compliance for the patient. However, the loading of BC with lipophilic substances generating a drug delivery system is still challenging due to the hydrophilicity of the material [4]. To overcome this challenge and to achieve lipophilic drug incorporation and a controlled release, *post-* as well as *in situ-*modifications of the biopolymer during the bioprocess were investigated. Native BC was synthesized by the bacteria strain *Komagataeibacter xylinus* at 28 °C in trays (20 x 20 cm) for seven days. For *in situ-*modification of the biopolymer BC during the cultivation in Hestrin-Schramm-medium different additives (e.g. with poly(ethylene glycol)) were tested. As a main result, pore sizes of the cellulose network could

be varied in the range of 2 – 10 µm. *Post*-modification strategies focused on a hydrophobization of the nanostructured BC network in order to increase the uptake of lipophilic drugs. Results of the successful BC modification by acetylation or oxidation with 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) and subsequent conjugation with more hydrophobic compounds such as phenylalanine will be presented. An in-vitro toxicity test (MTT assay) proved preserved biocompatibility of the chemically modified biomaterial. In case of both, *in-situ* as well as *post*-modification, different loading capacities for anti-inflammatory model substances of varying lipophilicity (e.g. diclofenac & indomethacin) and release profiles could be observed. These findings are currently used for the incorporation of lipophilic frankincense extract, quantified by the lead substances 3-O-acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA) into BC for innovative product design based on highly promising natural compounds.

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POS.150

In situ aggregation of liposomes as a novel injectable depot system – characterisation of the drug delivery system and encapsulation of a local anaesthetic

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Compared to conventional parenteral formulations, injectable depot formulations owing to a sustained drug release offer several advantages, such as a reduced dosing frequency - and consequent improved compliance - or a predictable release profile. Additionally, fluctuations in the drug blood level could be smoothed and thereby side effects reduced [1].
Different drug delivery systems based on polymers or lipids are used to form injectable depot formulations enabling release kinetics from days to months. Controlling the aggregation of negatively charged liposomal formulations could be used to induce a depot formation of a new injectable, long-acting drug delivery system. Although the aggregation behavior of negatively charged phospholipids (NCP) was already described [2,3], aim of our study is to investigate its use as depot formulation in a detailed manner.
A series of NCPs with glycerol-, serine- or phosphatic acid head groups were mixed in different compositions with the zwitterionic phospholipid L-α-phosphatidylcholine (EPC). Liposomes were prepared by film hydration method and subjected to freeze-thaw-cycles. Upon extrusion, homogeneous large unilamellar vesicles with a mean diameter of 150 nm were obtained, as measured by dynamic light scattering. The extent of aggregation in presence of calcium or magnesium, assessed by turbidity measurement (OD_{400}), suggested that the aggregation profile of the tested formulations is dependent on the nature of the phospholipid head group and the linked acyl chain. Particularly, EPC formulations with distearoylphosphatidylglycerol (DSPG), dipalmitoylphosphatidicacid (DPPA) and dioleoylphosphatidic acid (DOPA) showed a noteworthy aggregation tendency [4].
Their potential as drug delivery system was investigated by encapsulating the weak base bupivacaine by means of an active loading method. Different conditions were tested and depending on the used NCP encapsulation efficiencies up to 60% with final drug-to-lipid ratios up to 0.1 were achieved using an ammonium gradient.
For stabilizing the formulations during storage and avoiding either hydrolysis and oxidation of the phospholipids or drug leakage, a freeze-drying method was established. Sucrose in sugar-to-lipid ratios of 10:1 and 15:1 enabled to stabilise the bupivacaine-loaded liposomes during freeze-drying and subsequent reconstitution, as proven by negligible changes in liposomal size and polydispersity index.
The *in vitro* release profile of bupivacaine from the aggregated liposomes is currently being investigated in order to proof the potential of the designed delivery system as an injectable depot for sustained drug release.

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POS.151

Premix membrane emulsification: Is it possible to obtain nanoemulsions with particle sizes below 100 nm?

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Nanoemulsions are being intensively studied as carrier systems for poor water soluble drugs. Premix membrane emulsification is a promising method to produce these carriers with small particle sizes and narrow particle size distributions. In a previous study, small particle sizes, however usually larger than 100 nm, were achieved using different membrane materials [1]. The present study addressed the question whether it was also possible to obtain stable nanoemulsions with particle sizes smaller than 100 nm.
An instrumented small scale extruder was used for preparation [2]. A coarse pre-emulsion dispersed with an Ultra-Turrax was processed 27 times through Anodisc® membranes (d= 41 mm, pore size 0.2 µm, 0.1 µm, 0.02 µm). Anodisc® membranes consist of aluminium oxide with defined pore size and high porosity, narrow pore size distribution and high hydrophilicity. The hydrophilicity leads to a good wettability (without use of wetting agents) which is important for the successful preparation of O/W emulsions [1].
The emulsions consisted of 10 % Miglyol® 812 (MCT) and various concentrations (1 %- 30 %) of sucrose laurate as emulsifier in double distilled water. Sodium azide (0.05 %) was used to preserve the emulsions during storage under nitrogen at 20 °C. Resulting particle sizes and particle size distributions were determined using a laser light diffractometer with polarization intensity differential scattering technology (PIDS) or photon correlation spectroscopy (PCS).
The influence of the membrane pore size on the particle size of the emulsion was investigated and the particle size to pore size ratio calculated. The ratio increased with decreasing pore size indicating a decrease in the effectivity of droplet size reduction. Mean particle sizes below 100 nm were only achieved with 0.02 µm Anodisc® membranes. Therefore, only this type of membrane was used in further experiments.
In these experiments, sucrose laurate concentrations from 1 % to 10 % were applied to identify the minimal required sucrose laurate concentration to obtain particle sizes in the target range. The minimal required sucrose laurate concentration was located around 1.5 %. In samples with 1 % or less sucrose laurate particles below a mean size of 100 nm could not be obtained.
The presence of an excess of emulsifier may accelerate Ostwald ripening [3].Thus, the particle size of samples with 1% to 10% sucrose laurate was monitored over 12 weeks. There was indeed an increase in particle size especially for emulsions with higher sucrose laurate concentrations. At the same time, the particle size distribution became narrower the polydispersity index decreasing slightly. A linear relationship between the Ostwald ripening rate and the sucrose laurate concentration could be observed. At the end of the monitoring time, sucrose laurate concentrations above the minimal required concentration up to 3 % had led to minor particle size growth (< 10 %) and still yielded particle sizes below 100 nm.
In conclusion, nanoemulsions with particle sizes below 100 nm could be prepared by premix membrane emulsification using Anodisc® 0.02 µm

membranes and sucrose laurate as emulsifier. Long-term stability could be achieved by using sufficient but preferably low emulsifier concentrations.

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POS.152

Polymer stabilised emulsions with enhanced substantivity

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Topical formulations are the most common way to treat skin diseases. Thereby the site of action can be located on the skin surface or within the skin. In practice, a substantial amount of the applied formulation will be removed by contact with other surfaces, such as clothing or other skin areas [1]. To counterbalance this and to achieve the desired therapeutic outcome, higher drug concentrations or shorter application intervals are needed. But this results in undesired effects or poor compliance. Higher consumption of the preparation and pollution of the patient's environment with active pharmaceutical ingredients are other occurring problems. By improving the substantivity of the formulation a higher amount of formulation and thus active will remain on the skin surface. Positive effects on efficiency and safety can be expected. Formulations with liquid, semi-solid or film-forming character show substantial differences in their substantivity [1, 2]. Thus tuning the rheological behaviour is a promising approach to obtain preparations with enhanced substantivity.
We therefore developed topical formulations with different rheological characteristics. The developed polymer stabilised formulations differ in their rheological behaviour pre and post application to the skin. The formulations differed in oil phase and polymer concentration. Various additives and their effect on the rheological behaviour were investigated. Two suitable ex-vivo testing methods [1, 2] proved the high substantivity of the formulations. Almost the complete applied formulation remained on the skin surface after skin-to-skin or clothing-to-skin contact. In ex-vivo permeation experiments using Franz diffusion cells clothing-to-skin contact was simulated and compared to control samples. No differences were found, neither in permeation rate nor in permeated amount.
The developed formulations showed improved substantivity compared to conventional dermal preparations. Benefits in efficiency and safety but also ecological and economic aspects can be anticipated. The presented work is a promising concept to improve the therapy of skin diseases.

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POS.153

Mucoadhesive granulation and compression of nanoparticles

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Nowadays the most new chemical entities have low solubility and permeability properties and are part of class IV of the biopharmaceutical classification system (BCS). A development of a peroral dosage form with those drugs is a big challenge. However there are different ways to improve the bioavailability of those drugs.
An interesting approach is to embed the drug in nanoparticles in order to improve the transport across the gastrointestinal barrier. The formulation of nanoparticles in form of a solid dosage form can be achieved by using fluid bed granulation followed by compression as previously shown by Horster (*1*).
For drug targeting in the small intestine the usage of mucoadhesive excipients is a very promising approach. The mucoadhesion can enable a regiospecific sustained absorption of drugs or drug-loaded nanoparticles by the target cells.
In this study, both approaches were combined to develop a mucoadhesive nanoparticle based solid dosage form.
A mucoadhesion measurement method was developed (2) and a screening of different mucoadhesive excipients was performed. The best mucoadhesive excipients Noveon AA-1 and Carbopol 971P NF were used for fluid bed granulation of polymeric nanoparticles. The obtained granules were blended and compressed with a rotary press. Different compression settings were used in order to investigate the influence on the nanoparticle release by the tablets. The nanoparticle release by the obtained mucoadhesive granules and tablets was analysed regarding particle size and yield by using the Zetasizer Nano-S.

Acknowledgments

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POS.154

TEM of novel colloidal microstructures of ß-escin, DPPC, and cholesterol and the influence of ß-escin on stratum corneum and keratinocytes

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High interaction potential of plant saponins with cholesterol has long been known [1] and was suggested as reason for cytotoxic effects of saponins on viable cells [2]. From the latter several questions arise: Does saponin solubilize cholesterol or other membrane constituents to form colloidal structures different from liposomes and appropriate as drug carriers? And, does saponin affect drug permeation upon dermal application?
ß-Escin from horse chestnut (*Aesculus hippocastanum L.*) has long been used in dermal preparations for vein weakness because of its anti-edematous, anti-inflammatory and venotonic properties [3]. It is a triterpene saponin with one sugar chain (monodesmosidic). Both ß-escin and cholesterol have a large hydrophobic backbone with a hydrophilic group at C-3 and share poor solubility in water. In this study, ß-escin was investigated with regard to colloidal particle formation with cell membrane components (dipalmitoylphosphatidylcholine (DPPC), cholesterol) and visualized with TEM (transmission electron microscopy). Furthermore, the influence of ß-escin on the viability of keratinocytes was studied with an MTT-assay as well as its interaction with isolated human stratum corneum (SC). SC is mainly composed of cholesterol, ceramides and free fatty acids representing the main barrier for the dermal absorption of drugs.
TEM of an aqueous pseudo-ternary system (ß-escin, dipalmytoylphosphatidylcholine (DPPC), cholesterol), prepared using the lipid film method, showed new colloidal structures with a size of about 100-200 nm built up from ring-like and worm-like subunits [4].

Interestingly, colloidal structure formation was also observed in the absence of DPPC which suggested cholesterol as the essential interaction partner of ß-escin. The latter was confirmed in Langmuir monolayer studies with regard to intercalation of ß-escin into a cholesterol monolayer [4]. Since previous studies did not address the effects of ß-escin on keratinocytes of the epidermis, MTT-assays were performed with different concentrations of ß-escin on monolayers of HaCaT cell line for 2h and 24h, respectively. The IC50 value was about 0.04 mg/mL after 2h and about 0.03 mg/mL after 24h. Even low concentrations of ß-escin led to a decreasing metabolic activity of the cells, which in turn might be due to solubilization of cholesterol by ß-escin. To confirm the results from the MTT-assay, the influence of an aqueous ß-escin solution on isolated SC was examined. Suspensions of isolated SC either in pure water or in an aqueous ß-escin solution were slowly stirred over a period of 5h. Subsequently the aqueous phases (with and without ß-escin) were examined with TEM. The results showed nanoparticular structures only in the presence of ß-escin, which is possibly due to an interaction between ß-escin and SC components. Whether SC permeability for drugs is affected by the pretreatment with aqueous ß-escin solution will be studied in the near future.
In conclusion, ß-escin is a saponin with promising properties with regard to colloidal particle formation in the presence of cholesterol alone or from isolated SC. Such colloidal systems are suggested as potential drug delivery systems. Furthermore, the solubilization of cholesterol by ß-escin may affect drug permeation through the skin.

Acknowledgments: The supply of DPPC from Lipoid GmbH (Ludwigshafen, Germany) is gratefully acknowledged

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POS.155

Miscibility study of phospholipids with oils and fats for the liquid-filling of hard capsules

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A large number of new drug candidates are poorly water soluble. Phospholipids are interesting excipients for these substances because of their solubilizing properties and their biocompatibility. However, they are not widely used for solid oral formulations yet. With regard to oral administration, they can for instance be formulated as filling mass for hard capsules. Filling the mixtures in the liquid state with subsequent solidification is desired to avoid leakage from the capsules. For this purpose, melting fats can be utilized to prepare thermosoftening formulations, whereas thixotropic oleogels liquidify due to shear stress. A systematic miscibility investigation was conducted to find suitable phospholipid-fat/oil combinations as base for such mixtures.
In the present study, Phospholipon® 90 G and Lipoid® S LPC 80 were tested with rapeseed oil, Miglyol® 812, Witepsol® W 35 and coconut oil. Phospholipon® 90 G contains at least 94 % phosphatidylcholine, while Lipoid® S LPC 80 is comprised of at least 80 % monoacyl-phosphatidylcholine. Both phospholipids are obtained from soybean and therefore have a high fraction of unsaturated fatty acids.
To prepare the mixtures, the phospholipids were dissolved in an excess of ethanol at 60 - 70 °C. Fats or oils were added and stirred at 60 °C until the mixture appeared homogeneous. Finally, the excess of ethanol was removed by rotary evaporator to a final concentration of 5 % ethanol. The mixtures were investigated macroscopically, by polarizing microscopy and wide-angle x-ray diffraction.
Rapeseed oil and Miglyol® 812 yielded microscopically homogeneous and isotropic mixtures with up to 70 % Phospholipon® 90 G (figure 1). Further increasing the fraction of phospholipid to 80 % led to a firm skin on the surface of the formulations rendering the mixture inhomogeneous. When mixing Phospholipon® 90 G and coconut oil or Witepsol® W 35 particles were observed when investigated under the polarizing microscope. According to the reflections in the wide-angle x-ray diffractograms, these particles consisted of the respective fats.
Miglyol® 812 and 10 - 50 % Lipoid® S LPC 80 resulted in turbid but homogeneous mixtures. When viewed under the polarizing microscope anisotropic structures and maltese crosses were visible (figure 2). Reflections in the x-ray diffractogram were correlated to the crystalline phospholipid. Mixtures of Lipoid® S LPC 80 and rapeseed oil were macroscopically inhomogeneous. The formulations of Lipoid® S LPC 80 with coconut oil and Witepsol® W 35 exhibited microscopic particles. The reflections in the diffractograms of the mixtures were related to Witepsol® W 35 and Lipoid® S LPC 80.

Figure 1: 70 % Phospholipon® 90 G and 30 % rapeseed oil

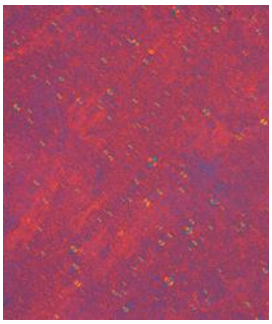

Figure 2: 50 % Lipoid® S LPC 80 and 50 % Miglyol® 812

Homogeneous mixtures were thus obtained when mixing up to 70 % Phospholipon® 90 G with rapeseed oil or Miglyol® 812. These combinations can be used for the formulation of thixotropic oleogels. Mixing 10 - 50 % Lipoid® S LPC 80 with Miglyol® 812 yielded semisolid turbid formulations which might also be used in oleogels. Formulations of Phospholipon® 90 G and Lipoid® S LPC 80 with Witepsol® W 35 or coconut oil were macroscopically or microscopically inhomogeneous. Therefore, they do not appear suitable for the intended purpose.

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POS.156

Contact-active antibacterial polymers: materials for packaging and clinical hygiene

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Biofilm formation, the accumulation of unwanted accumulation on a surface, is a major issue for almost any type of material used in clinical hygiene[1] or packaging industry.[2] Current approaches against biofouling are modification with hydrophilic substances such as polyethyleneglycol to build up highly hydrated surfaces to improve biopassivity against proteins and microorganisms.[3] A particularly promising approach to prevent biofilm formation is the immobilization of polycationic polymers on surfaces to create contact-active materials.[4] Those antimicrobial agents interact destructively through electrostatic attraction with the negatively charged bacteria membrane, which results in cell death.[5] Herein, we present the surface modification of polyethylene foils *via* plasma-induced polymerization of quaternary ammonium salts. The activation with atmospheric air plasma generates functional groups on to the polyethylene surface, which can be used afterwards with antibacterial monomers for graft polymerisation. Polyethylene foils modified with this method exhibit excellent antibacterial spectrum against gram-positive and gram-negative bacteria.

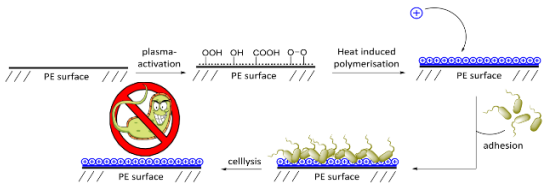

Fig. 3: Schematic overview of the applied modification process for polyethylene foils. Atmospheric air plasma activation leads to generation of oxygen containing functional groups, which can be used for graft polymerisation of suitable quaternary ammonium salt monomers.

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POS.157

Fluoroquinolone ionic liquids with improved solubility and antibiofilm activity

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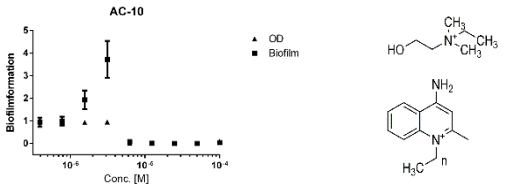
Biofilm-growing bacteria are responsible for a number of clinically relevant infections. These are difficult to treat due to the increased resistance to antibiotic therapy that is largely mediated by the bacterial biofilm: The lowered metabolic rate, the higher frequency of gene mutation and the diffusion barrier formed by the biofilm itself are critical factors that impede effective clearance of the bacterial infection. [1] Despite the fact that several substance classes with antibiofilm properties are well known, the combination of antibiofilm agents with established antibiotics remains a challenge and is the motivation of this work. Therefore a set of choline and aminoquinaldine derived substances with putative antibiofilm activity was synthesized and subsequently tested against biofilm forming strains of S. aureus. While the choline analogues were found to be inactive against S. aureus, the dequalinium derived compounds showed antimicrobial and antibiofilm activity with minimal inhibitory concentrations of 50 µM.



These quarternary ammonium cations were then used for salt formation with commonly administered fluoroquinolone antibiotics such as ciprofloxacin and norfloxacin. Due to the bulkiness of the counterions the resulting salts are mainly amorphous ionic liquids with higher kinetic solubility compared to the free acid. As the counterion in these ionic liquids not only serves to improve the solubility behaviour of the compound but also imparts an additional pharmacologic activity, this work can serve as proof-of-concept for a fourth generation of pharmacological ionic liquids. Hereby the instrument of salt formation is not only used to tailor the physicochemical properties of the drug but also to complement its pharmacodynamic action. [2]

On the basis of these promising initial results studies are ongoing on a diverse group of bacteria with the intent of demonstrating a synergistic effect of antibiotic and counterion on biofilm formation and antimicrobial activity.

Acknowledgments: This work was supported by the Bayrische Forschungsstiftung [grant number AZ-1204-16]

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POS.158

Redox-sensitive nanogels: A promising approach for efficient drug delivery in mucosal tissue?

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Mucosal surfaces in the human body are robust barriers comprising not only a physical but also a chemical barricade against foreign substances and pathogens. These epithelial surfaces are mainly coated with the dynamic and tenacious mucus gel, which protects the underlying epithelia and renders mucosal tissues their obstructive potency by its trapping and rapid clearance mechanism through steric hindrance or adhesion interactions [1]. Innovation and progress in nanotechnology throughout the recent years presented various nanocarrier systems for topical drug delivery to different mucosal tissues [2]. Nevertheless, the variation between the biological properties of targeted mucosal tissues, in addition to different chemical properties of the intended drug-cargos for encapsulation, still requires further developments and new delivery strategies. Hence, efficient trans-mucosal drug delivery remains a challenge, especially for epithelia with thick mucus layers, such as the intestinal mucosa [3]. For this purpose, our project presents novel redox sensitive poly glycerol and poly(N-isopropyl methacrylamide) based nanogels formed through disulfide bridges (pNIPMAM-S-S-dPG) as potential nanocarriers for the treatment of inflammatory bowel disease by delivering, for example, anti-inflammatory drugs to the human intestine. In this context, these nanogels were investigated in terms of native intestinal mucus gel interactions, mucopenetration to freshly excised jejunum, biocompatibility, and internalization to the tissue underlying immune cells, i.e. human macrophages.
To investigate the mucus related biodegradation, native intestinal mucus gel was isolated from sacrificed pigs and incubated with the nanogels at 37°C for 1, 3, 6, 12, and 24 hours. Subsequent gel permeation chromatography (GPC) studies demonstrated a gradual reduction of the pNIPMAM-S-S-dPG in mucus gel over time with 98% degradation after 12 hours, compared to control studies using pNIPMAM-dPG nanogels without the disulfide bonds, which showed by the same conditions no biodegradation in mucus. Additionally, parallel control degradation studies were also carried out for the nanogels with the reduced glutathione (GSH) at its reference concentration in the normal intestinal mucosa [4]. Mucopenetration into the intestines was also studied ex-vivo in freshly excised porcine jejunum. The explants were treated with the pNIPMAM-S-S-dPG and pNIPMAM-dPG nanogels on a Franz-cell setup at 37°C for 3 hours. Resulting fluorescence microscopy images of the rhodamine B-labelled nanogels showed mucopenetration, compared to almost complete clearance of the pNIPMAM-dPG nanogels by the secreted mucus gel. These nanogels were also studied in vitro with primary human macrophages cultured from monocytes that were initially isolated from whole human blood. Both nanogels demonstrated high biocompatibility to macrophages following 24 hours treatment in addition to successful active cell-internalization capacity within 3 hours of treatment.
Our findings so far, declare a highly biocompatible drug delivery system, capable of surpassing the rapid clearance mechanism of the intestinal mucus gel and infiltrating its complex matrix. These properties demonstrate promising nanocarriers for effective anti-inflammatory drugs

encapsulation and further investigation of treatment efficiency in 3D human intestinal equivalents with a Crohn disease-like inflammation.

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POS.159

smartLipids – submicron industrial concentrate of phenylethyl resorcinol for skin whitening

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Phenylethyl resorcinol (PER) proofed to be one of the most efficient cosmetic whitening actives [1] but the exposure to UV light leads to a quick chemical degradation of PER, indicated by a pronounced discoloration. The encapsulation of PER into lipid nanoparticles improves the chemical stability and overcomes its poor aqueous solubility [2]. Furthermore, the in-vitro efficacy of PER was tested to be distinctly increased when incorporated into lipid nanoparticles [3]. Aim of this study was to develop a formulation sufficiently high concentrated in particle concentration, and with high PER loading to be used as "industrial concentrate" in the production process of cosmetic products.
As the 3rd generation of lipid nanoparticles, smartLipids are characterized by higher loading capacity compared to SLN or NLC. This can be achieved by using chaotic lipid mixtures being chemically composed of many single components – either using blends of up to 10 single lipids or by using natural lipids that natively contain various constituents. As a consequence from the chaotic lipid structure, there are no/little polymorphic transitions in smartLipids [4]. This allows higher loading without an expel of the active from the lipid matrix by storage-induced transformation to higher-ordered lipid modifications.
For PER smartLipids, a mixture of C10-C18 triglycerides was used as lipid matrix. The formulation was produced by pre-emulsifying with a rotor-stator-homogenizer (Ultra-Turrax, IKA-Werke, Germany), followed by hot high pressure homogenization (Micron LAB 40, APV, Germany) at 80°C and 500 bar. The formulation was composed of 5% lipid matrix, 5% PER, 1.4% emulsifying cetaryl alcohol and 88.6% water. The product shows a mean particle diameter of 110 nm (z-ave, dynamic light scattering) and a narrow particle size distribution, indicated by a polydispersity index of 0.15. These results are in good agreement with the laser diffraction measurements, showing a median particle diameter of 140 nm and a D99%-diameter of 260 nm. No aggregates or micrometer-scaled particles were detected under microscopic observation. The particle size and distribution remained unchanged after 6 months of storage at room temperature. The encapsulation efficacy of PER was determined to be 94%, being higher or as high as competitive formulations in literature [2,3] and indicating a firm inclusion of PER into the smartLipids matrix. Thus, a good chemical stabilization of PER can be predicted.
The loading capacity for PER was increased from 20% in NLC in literature [2] to 50% in smartLipids, still yielding a particle concentrate being physically stable for 6 months. Interestingly, solubility studies of PER show that only 33% PER can be diluted in the lipid matrix. Thus, the smartLipids matrix tolerates a certain amount of "overloading": Further undissolved active can be immobilized in deliberate flaws and imperfections in the lipid structure, being a result of the chaotic composition of the smartLipids matrix.
Presently, the effective PER concentration is about 0.5% in the traditional cosmetic dermal products. The efficacy of PER in lipid particles is distinctly increased [3], thus a lower concentration of about 0.1% in products should be effective. The developed formulation can thus be diluted by a factor 10-50 in industrial production, a dilution factor sufficiently high as industrial concentrate.

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POS.160

Tablets made from paper – first attempts for large-scale production

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Introduction: In a recent study it was shown that paper can be utilized as a drug delivery system to improve the solubility and dissolution rate of poorly soluble actives, by loading actives into the pores of the paper in amorphous state [1]. Another study revealed that paper can be transferred into tablets without further excipients, yielding to tablets with acceptable pharmaceutical quality and an appearance being similar to classical tablets obtained from powder or granules [2]. Until now tablets made from paper were produced manually by cutting paper into small pieces, which were manually placed into the cavity of the tablet press. The production technique is suitable for small batches in lab-scale but not suitable for large-scale production.
Aim: Therefore, the aim of this study was to develop a method for the production of tablets made from paper in large-scale.
Materials and methods: The study was performed in two steps. In the first step paper (Tempo®, Essity, Sweden) was transferred into pellets and in the second step pellets were transformed into tablets by using a single punch tablets press (EK0, Korsch, Germany) in continuous mode. Pellets were characterized in regard to shape, size, size distribution and flowability. Tablets were characterized according to the requirements of the European Pharmacopeia 8.0.
Results and discussion: After optimization, spherical pellets with a size of about 1-3 mm were obtained by shredding the paper into flakes and by granulation of the flakes with water in a high shear mixer and subsequent shaking on a shaking plate (Fig. 1 A-C). Tablets from pellets could be obtained manually and in continuous mode. The tablets appeared ~~nice and~~ with a shiny surface (Fig. 1D). However, in contrast to tablets made from paper in "direct compression" not all pharmaceutical criteria could be met by the tablets made from paper pellets. Especially mass uniformity, resistance to crushing and friability were not fulfilled. Reasons are probably the elasticity of the paper pellets and the large content of incorporated air in the pellets, which leads to elastic, soft pellets with inhomogeneous air content. Consequently, the next step aimed at reducing the elasticity of the pellets. For this purpose, pellets were produced by sucrose solution as binder, sucrose is known to improve the plastic deformation properties of powders. These pellets could then be transferred into tablets with acceptable pharmaceutical quality, e.g. sufficient hardness, friability and disintegration time.

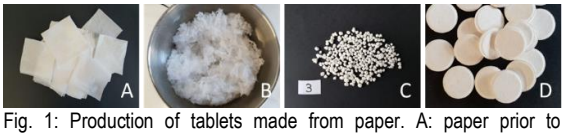

Fig. 1: Production of tablets made from paper. A: paper prior to processing, B: production of pellets, C: pellets made from paper, D: tablets made from paper pellets and with continuous production mode.

Conclusion: Tablets with pharmaceutical quality can be obtained from ordinary paper. Large-scale production is possible by transferring the paper into pellets prior to the tabletting process.

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POS.161

Dermal drug delivery with nanocrystals – Influence of skin condition and massage on the penetration efficacy

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Introduction: Nanocrystals are composed of 100% active and possess a size below 1µm. Due to their small size, the physico-chemical properties are changed when compared to bulk material. Nanonisation offers an increase in dissolution velocity as well as kinetic solubility, thus nanocrystals can be used to increase the bioavailability of poorly soluble actives via enhanced passive diffusion trough membranes. The nanocrystal effects can be exploited for many applications, i.e. dermal drug delivery [1]. In previous studies the influence of nanocrystals size and penetration enhancer on the penetration efficacy of the active from nanocrystals could be shown [2]. Until now, the influence of skin conditions and application parameters remained undetermined. Therefore, the aim of this study was to investigate the influence of skin condition and massage on the dermal penetration efficacy of actives from nanocrystals.
Materials and methods: Hesperetin was used as a model drug. It is a natural flavonoid with high anti-oxidative properties, poor water solubility and lipophilic character. Hesperetin nanocrystals were produced by using small scale bead milling and subsequent high pressure homogenization [2]. Nanocrystals were applied as aqueous nanosuspensions to either wet or dry porcine ear´s skin and with or without a short massage. After a penetration time of 30 min skin penetration was studied by classical tape stripping followed by HPLC analysis.
Results and Discussion: The penetration of the active was slightly more efficient when the formulations were applied to dry skin. Results can be explained by the theory that the stratum corneum cells swell by absorbing water and thus the penetration of lipophilic actives like hesperetin decreases. Unexpectedly, massage was found to decrease the penetration efficacy, i.e. when nanocrystals were applied to the skin with massage, the total uptake of the active into the skin was about 3fold lower. A possible explanation is the increased water content in the skin upon massage, which hampers the penetration of the lipophilic active. However, more investigations are necessary to understand this phenomenon in more detail.
Conclusion: Skin condition and application parameters influence the dermal penetration of actives from nanocrystals. Data until now indicate, that the most efficient penetration can be achieved by applying the nanocrystals to dry skin and without massage.

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POS.162

Approach of a Physiologically-Based Permeation Model Utilizing LC-MS/MS to Investigate the Safety Potential of Buccal Drug Applications

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Introduction:
Dosage forms for the oral cavity are achieving certain market popularity through the high acceptance by their patients and the suitability of the buccal membrane as a pathway for direct local treatment and as an alternative systemic application way for drugs with a low oral bioavailability. However, intoxications caused by administration in the oral cavity are reported leading to life-threatening side effects (e.g. lidocaine hydrochloride [LDC-HCL]) [1] and emphasize the need for safe innovative intraoral formulations. Physiologically-based ex-vivo permeation studies can support the description of permeation behaviour of drugs and allow the required development of safe innovative intraoral formulations to avoid intoxications and ensure efficiency. Current studies describe the processes after application only to a certain extent with physiological reference. The aim of this study was the establishment of physiologically-based permeation studies through LC-MS/MS by development of a sensitive analytical method adapted on clinical relevant short-term applications (<30 min). This included investigations with different physiological buffer systems for the permeation chambers and with fresh and frozen membranes on permeability examinations of LDC-HCl and TCA.
Materials and Methods:
The permeation of LDC-HCl and triamcinolone acetonide [TCA] were investigated by applying oesophageal porcine membrane [2] with 300 µm thickness being best comparable to human tissue. Permeation studies were conducted utilizing an automated permeation apparatus (Kerski cells) with an area of 0.82 cm^2. As physiological buffer systems dipotassium hydrogen phosphate and ammonium bicarbonate adjusted at pH 6.9 for the donor side and at pH 7.4 for the acceptor side with constant stirring (350 rpm) at 34 °C were used. The samples were quantified by high performance liquid chromatography coupled with tandem mass spectrometry (LC-ESI-MS/MS). The permeation profiles were investigated through frozen (stored at -80 °C in isotonic saline solution) and freshly prepared membrane with single and simultaneous API solutions.
Results:
Monitored for one hour, the permeation of LDC-HCl (200 µl/mL) through frozen and fresh membrane [Fig. 1] as well as in bicarbonate and hydrogen phosphate buffer systems showed no significant difference. Lidocaine showed a fast permeation after five minutes (first point of measurement). Permeability of lidocaine is not influenced by the presence of TCA (J = 27.4 and 25.8 ng/cm^2/min [single vs. combined application]) which enables the combined administration for synergistic treatment of oral diseases. TCA as a lipophilic drug showed a slow-onset with a relevant permeation after one hour, which indicated retention in the epithelium and required investigations by membrane extraction studies.

Figure 1. Permeation of lidocaine, mean ± sd, n = 4 (fresh) and 8 (frozen)

Conclusion:

Utilizing LC-MS/MS as determination method enabled the first attempts of development of a physiologically-based permeation model, which allowed the evaluation of clinical concentrations in adaptation to short-term application. The model will be developed further to incorporate additional physiological aspects. In combination with membrane extraction studies a relevant and detailed description of the permeability of TCA is possible.

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POS.163

smartPearls – stabilization of amorphous actives for improved dermal delivery

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New dermal actives and drugs being developed recently show promising action on forgoing in vitro tests but almost no effect when applied on human skin. The insufficient dermal bioavailability mostly results from the low solubility of these actives. One established approach to increase the solubility without changing the actives chemical structure is the production of nanocrystals, proved by the increasing number of nanocrystal products on the pharma market. Contrarily, the use of an active in its amorphous state is a well-known approach but the practical implementation is challenging due to re-crystallization tendency.

smartPearls are a new dermal delivery system, combining both approaches successfully. They consist of spherical silica matrices (3-10 µm in diameter) with tiny pores (2-50 nm) in which actives can be incorporated amorphously. Not enough space is given for re-crystallization, thus amorphous state can be long-term preserved.
Solubility studies of brick molecules being insoluble in both phases of dermal vehicles (e.g. water and oil phase of cream or lotion) were performed. Hesperidin smartPearls for example showed a 10-times increased water solubility compared to hesperidin raw drug powder (µm-sized and crystalline) and almost 5-times higher solubility compared to hesperidin nanocrystals (nm-sized and crystalline). Rutin, also a brick molecule with anti-oxidant property, were formulated as smartPearls in a hydrogel. Its penetration was measured on porcine ear by a tape stripping test. Up to 7-times higher rutin concentrations were observed compared to corresponding nanocrystal hydrogel. Especially, rutin penetration was pronounced for the deeper layers of the stratum corneum represented by the strips 10-20.
Storage stability investigations at room temperature proved that the amorphous state in porous silicas can be preserved for up to 5 years. Thus, in terms of physical stability, smartPearls are market feasible. Also the fact that the incorporation of smartPearls into the final dermal vehicle can be realized by simple admixing supports their market feasibility. After loading, smartPearls have high densities. To guarantee a homogeneous distribution of them in vehicles, -carrageenan can be added into the vehicles water phase, generating viscoelastic properties. 0.5 % -carrageenan for example stabilized 50 µm large smartPearls at increased g-force of 40. Nonetheless, rheological behavior of vehicles stayed almost unchanged.

Considering all these properties, smartPearls are a highly promising dermal delivery system for poorly soluble actives.

POS.164

Enhanced Cell Recognition of Heteromultivalent Nanoparticles using a Biomimetic Approach

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Having been in focus of drug delivery research for over a decade, the field of nanomaterials had to face some backlashes in recent years. For instance, it was shown that bioavailability of nanoparticles (NPs) in solid tumors was only around 0.7% [1]. Since cell-particle interaction plays a pivotal role in increasing target specifity [2], it is crucial to explore innovative ways to enhance cell recognition. Our approach is to create nanomaterials with the ability to mimic viral infiltration strategies. Viruses often rely on various surface components binding simultaneously or sequentially to their target sites on the cell surface [3]. Many of them thereby comprise structures that facilitate cell attachment and elements that promote subsequent internalization [4]. Consequently, our goal was to design polymer-based nanoparticles carrying one ligand for target cell identification and a second ligand for affiliating receptor-mediated endocytosis. Being a key player for glomerular homeostasis and contributing to several kidney pathologies like diabetic nephropathy [5], mesangial cells served as a model target. We used the highly expressed Angiotensin-II receptor type 1 (AT_1r) as initial binding site and the αVβ3-integrin as internalization mediator, since it had already been shown that its targeting using cyclo-Arg-Gly-Asp-D-Phe-Lys (cRGDfK) leads to effective nanoparticle endocytosis [6]. To that end, EXP3174, a losartan metabolite and potent AT_1r - inhibitor and cRGDfK were coupled to PEG-PLA block-copolymers via DDC/NHS and EDC/NHS chemistry respectively. Functionalized polymers were combined with PLGA to generate NPs by nanoprecipitation. Bifunctionalized particles efficiently bound to the mesangial AT_1r and thus inhibited Ca^{2+} influx following cell stimulation with angiotensin-II (AT-II). In contrast, non-functionalized NPs (control NPs) did not interact with the receptor, having no influence on AT-II dependent Ca^{2+} signal (Figure 1A). Moreover, NPs carrying both ligands showed a significantly higher mesangial cell uptake compared to control particles. Addition of free cRGDfK significantly decreased uptake, indicating that NP internalization was mainly caused by a NP - integrin interplay (Figure 1B). Showing great affinity for the mesangial AT_1 receptor and enhanced αVβ3-integrin-mediated cell uptake, our bifunctionalized NPs combine both characteristics of their attached ligands, making them a promising tool for highly specific mesangial cell targeting.

B

Fig 1. Cell culture experiments using rat-derived mesangial cells (A) Bifunctionalized NP showed high affinity for the AT_1 receptor, inhibiting Ca^{2+} influx upon AT II stimulation (IC_{50} = 53 ± 15 pM; Ca^{2+} - detection via Fura-2-AM). (B) Flow cytometry analysis indicated significantly enhanced uptake of bifunctionalized NP ($p < 0.001$).

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POS.165

Controlled Release of Therapeutic Nanoparticles for Systemic Drug Delivery

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Over the last decades nanoparticle (NP) based drug delivery systems have been explored extensively to improve drug therapies. However, a major drawback of NP based therapeutics for systemic drug delivery is their way of administration: the typically performed NP injections or infusions are a tremendous burden for patients since the application has to be repeated in short intervals over long treatment periods [1]. One way to overcome this problem could be the development of depot formulations, releasing the NP drug delivery system in a controlled way to reduce the number of injections necessary for disease treatment.
Hydrogels made of star-shaped polyethylene glycol (PEG) crosslinked by Diels-Alder chemistry have recently been identified as promising tools for the development of drug depots [2]. In this study we show that besides biologics also gold nanoparticles surface modified with SH-PEG_{2k}-COOH (GNP) can be embedded in such hydrogel matrices and that the particles are able to diffuse through the three dimensional PEG meshwork. The rate of particle diffusion seems to be correlated to the gels' average mesh size, since increasing release rates can be observed with decreasing polymer content or enhanced swelling and degradation of the hydrogel matrix (Fig. 1 A, B). To reduce the time lag until swelling and degradation allows for GNP release, different types of porogens were added to the hydrogel forming polymer mixture. The addition of non-functionalized star-shaped PEG or an emulsified dextran phase were found to create imperfections in PEG-crosslinking and therefore an earlier onset of GNP release (Fig. 1 C, D).

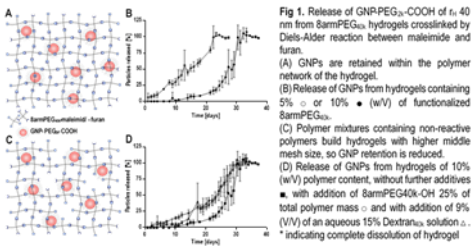

Fig 1. Release of GNP-PEG_{2k}-COOH of r_H 40 nm from 8armPEG$_{40k}$ hydrogels crosslinked by Diels-Alder reaction between maleimide and furan.
(A) GNPs are retained within the polymer network of the hydrogel.
(B) Release of GNPs from hydrogels containing 5% ○ or 10% ● (w/V) of functionalized 8armPEG$_{40k}$.
(C) Polymer mixtures containing non-reactive polymers build hydrogels with higher middle mesh size, so GNP retention is reduced.
(D) Release of GNPs from hydrogels of 10% (w/V) polymer content, without further additives ■, with addition of 8armPEG40k-OH 25% of total polymer mass ○ and with addition of 9% (V/V) of an aqueous 15% Dextran$_{40k}$ solution △.
* indicating complete dissolution of hydrogel

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POS.166

Virus-mimetic Nanoparticles for enhanced target-cell specificity

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Poor recognition of their target cells is currently a major drawback of nanoparticles (NPs). It leads to an insufficient accumulation at their target site, with bioavailabilites of as low as 0.7% [1]. To overcome this limitation specific hetero-multivalent ligand-receptor interactions have been investigated, which rely on an avidty gain of simultaneously presented ligands [2] by which an increased targeting specificity is achieved by addressing several different receptors [3]. Nevertheless, an issue that has been neglected by "conventional" hetero-multivalent NPs is that more cells in the organism can carry either one of the target receptors, leading to off-target NP loss and side effects. Here, we show an approach to increase NP target specificity by having them interact with their target cells in a virus-like manner. The viral binding and cell entry is usually a complex multi-stage process involving several recognition molecules [4]. Our NP design was inspired by influenza viruses, which require that their target cells activate hemagglutinin on their surface before binding to the secondary target molecule, responsible for cell-uptake [5]. We decorated block-copolymer NPs with angiotensin-I (Ang-I), a pro-ligand that initiates cell recognition by binding to the primary target molecule, angiotensin-converting enzyme (ACE). After this initial contact is established, the pro-ligand is enzymatically transformed to angiotensin-II (Ang-II), the active secondary ligand. Ang-II can bind the secondary receptor, angiotensin-II type 1 receptor (AT1R), confirming cell identity and triggering receptor mediated endocytosis [6]. By making the recognition process multi-stage, our virus-mimetic NPs achieved outstanding target-cell specificity when confronting them with different cell types (Figure 1A-B), while maintaining a strong receptor avidity.

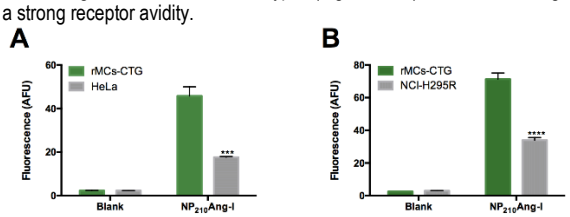

Fig 1. Virus-mimetic target cell specificity as investigated by flow cytometry using co-culure of target rat mesangial cells (rMCs) with off-target HeLa (A) or NCI-H295R cells (B). Ang-I modified NPs (NP_{210}Ang-I) were specifically taken up in rMCs avoiding HeLa and NCI-H295R cells, despite being the off-target cells in a ten-fold excess.

Acknowledgements: Financial support from the German Research Foundation (DFG), Grant Number GO 565/17-3, is gratefully acknowledged.

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POS.167

Nanoparticle albumin-bound technology – Drug properties as a crucial aspect for an application

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A lot of new developed drugs exhibit poor water solubility and benefit of formulating as nanoparticles. *Nab™ technology* (nanoparticle albumin-bound) is a nanoparticle manufacturing method without the use of synthetic excipients for stabilization. Additional adverse effects can therefore be avoided. These nanoparticles contain only the active pharmaceutical ingredient and the endogenous protein human serum albumin as a stabilizing agent. This results in lower allergy risks. Furthermore, natural transport ways of albumin can be used for drug targeting [1].
A prominent example is *Abraxane®* as an approved drug, which contains *nab™* paclitaxel manufactured by *nab™ technology*.
In this investigation two different model drugs were selected. Fenofibrate and Lumogen® Red were used to manufacture nanoparticles by *nab™ technology* and were compared to *Abraxane®*. The selection of the substances was made due to their poor water solubility and different molecular structures and weight. Fenofibrate represents a small molecule with low molecular weight, followed by paclitaxel and Lumogen® Red with the highest weight.
After nanoparticle preparation the morphology was characterized by scanning electron and atomic force microscopy (Fig. 1).

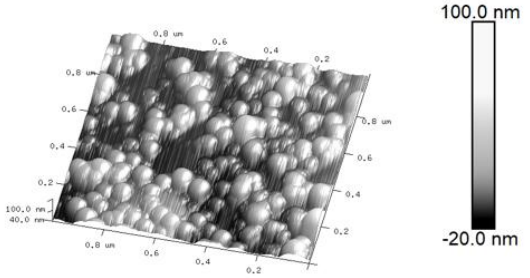

Figure 1: 3D height display of albumin stabilized Lumogen® Red nanoparticles by atomic force microscopy

Nab™ paclitaxel shows a very poor stability after reconstitution of the lyophilized product. Therefore, particular attention was paid to particle stability. The use of fenofibrate resulted in very unstable particles with insufficient physicochemical properties. Thus, it seemed to be an inappropriate drug for *nab*™ *technology*.
On the contrary Lumogen® Red could be formed into nanoparticles with a colloidal stability over several weeks and these particles were even more stable than *Abraxane®*.
In conclusion, the conducted experiments showed an influence of molecular structure and weight on the application of *nab*™ *technology*. A molecular interaction of the drug substance with albumin was probably necessary to produce stable particles using this method. Results of Lumogen® Red will be transferred to comparable lipophilic substances in further experiments.

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POS.168

Poly(vinyl methyl ether-alt-maleic anhydride) based nanoparticles and nanocapsules – An *in vitro* study under physiological relevant pH-values.

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Nano-based drug delivery systems are tools to improve the pharmacological profile and bioavailability of drugs. Developing an efficient system requires a better understanding of the interactions between particles and the body as well as the variables that have a significant impact on this interaction. Due to the ability to develop nonspecific adhesions to mucous membranes, Poly(methyl vinyl ether-alt-maleic anhydride) - PVM/MA (Fig. 1) in its native or cross-linked form, is considered to be particularly suitable as a polymeric matrix for nanoparticles for oral applications [1,2]. The presence of a maleic anhydride group gives to the polymer a moderate chemical reactivity, allowing grafting of the polymer backbone with primary amines and alcohols without the use of complex chemical reactions [3]. After administration, the polymer is expected to hydrolyze in an aqeous environment. The hydrolysis will change the microenvironment and leads to (i) the dissolution of nanoparticles or the (ii) the formation of nanohydrogels (for cross-linked nanoparticles). Unfortunately, no kinetics have been reported so far. Therefore, hydrolysis was investigated at different physiological pH values (1.2, 5.0 and 7.4) on plain and cross-linked nanoparticles. The results showed that dense polymeric nanoparticles are solubilized at different rates that are influenced not only by time but also by the pH of the medium. The most pronounced effect was observed at pH 7.4. Nanoparticle tracking analysis (NTA) and EPR spectroscopy demonstrated that the dense polymeric nanostructures were completely solubilized in less than 20 minutes (Fig. 1), while the cross-linked structure formed a mesh with hydrogel character. Therefore, the instability of PVM/MA nanoparticles in neutral pH hinders its application as a useful drug delivery system.
In addition, nanocapsules composed of PVM/MA and medium-chain triglycerides (PVM/MA-MCT NC) were developed and characterized. Plain and cross-linked PVM/MA-MCT NC were produced employing only acetone as an organic solvent and without the need for any type of stabilizers (Fig. 1). The stability of plain and cross-linked PVM/MA-MCT NC were also evaluated at physiological pH conditions. Dynamic light scattering (DLS) showed the pH of the medium did not change the particle size over time but had an important effect on the PdI of samples at pH 1.2. EPR spectroscopy also revealed the polymeric shell was affected by the environmental pH by the reduced mobility of the spin-probe at pH 1.2. The results suggested the polymer was partially hydrolyzed. The yielded amphiphilic polymer stabilizes the interface of the nanocapsules giving a stable structure, which is able to retain the model drug over time. Hence, comparing both PVM/MA colloidal systems, nanoparticles and nanocapsules, the nanocapsules offer a more protective environment for a lipophilic drug model than the nanoparticles, being a promising drug delivery system for oral administration.

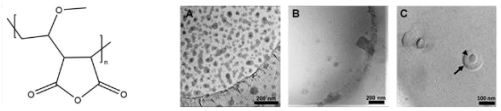

Figure 1. Chemical structure of PVM/MA copolymer. Cryo-TEM micrograph of (A) freshly prepared PVM/MA nanoparticles, and (B) PVM/MA nanoparticles in pH 7.4, 30 min after preparation. (C) FF-TEM micrograph of PVM/MA NC, arrow: polymeric shell; arrowhead: oil core.

Acknowledgements:

CAPES Foundation, Ministry of Education of Brazil, Caixa Postal 250, Brasília – DF, 70040-020, Brazil - CAPES scholarship Proc. nº 99999.010146/2013-00". Hause, G., Microscopy Unit, Martin Luther University Halle-Wittenberg, Weinbergweg 22, 06120 Halle, Saale, Germany. Meister, A.: Center for Structure and Dynamics of Proteins (MZP), Biocenter Martin-Luther-Universität (MLU) Halle-Wittenberg,Weinbergweg 22, 06120 Halle (Saale), Germany.

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POS.169

Formation of Colloidal Particles by Dialysis of Mixed Albumin Solution with Lecithin Emulsion: Incompatibility or Chance for a New Lipid-based Drug Delivery System?

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Dialysis is known as a common tool for purification. However, surprisingly the formation of colloidal particles with decreasing polydispersity index down to a monodisperse system has been observed during dialysis of a lecithin emulsion in combination with a human serum albumin (HSA) solution through a 3.5 kDa membrane.
These particles are considered as a new nanoscale drug delivery system due to their good biocompatible ingredients and the combination of lipid components with proteins, which could possibly be used for targeting applications. Dialysis as a particle forming method is a completely new, simple and highly scalable preparation technique for nanoparticles.
Due to the use of lecithin as emulsifying agent in parenteral formulations the mechanism of the particle formation was investigated.
For this the development of nanoparticles was monitored by photon correlation spectroscopy (PCS) measurements of particle diameter and polydispersity index (PDI) as a function of dialysis time. Furthermore, the dialysis of pure lecithin emulsions and HSA solutions showed no particle development, though a similar zeta potential.
The nanoparticle suspension was purified through a 300 kDa membrane to separate all components not participating in the nanoparticle formation. Afterwards size, PDI, and zeta potential were measured by PCS. The yield was determined gravimetrically.
Due to a lack of storage stability different strategies were tested to prevent the particles from further agglomeration, revealing polyvinyl alcohol (PVA) and poloxamer 188 as promising ingredients.
In conclusion a new nanoparticle system with a completely new preparation strategy was established and a theory about the particle formation was postulated.

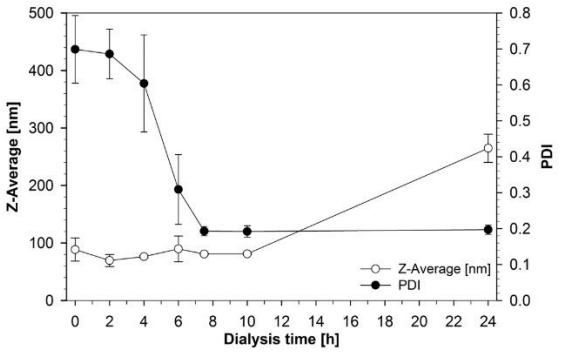

Figure 1: Formation of lecithin/HSA nanoparticles as a function of dialysis time

months of incubation. Increasing the GA content of the macromer further changed the properties of the scaffolds. With higher GA incorporation the initial compressive modulus was reduced and hydrolytic degradation was accelerated even more. For Tri134(LA2GA4)3.75, the compressive modulus was reduced to 9.2±2.9 MPa, and hydrolytic degradation of corresponding scaffolds was detectable immediately from the start of the experiment. Loss of more than 50% of initial mass was already observed after 4 weeks. Synthesis of macromers with an even higher comonomer feed of GA was not possible due to the insolubility of the products in organic solvents.
As illustrated by these results, it is possible to adapt the chemical composition of our three-armed macromers to control the mechanical (compressive modulus ranging from 9 to 30 MPa) and degradative (>50% mass loss after 4 weeks to 9-11 months) properties of macroporous scaffolds fabricated from the materials.

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POS.170

PST.08

Three-armed biodegradable material platform providing adjustable degradation properties

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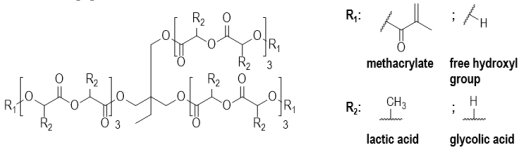
For tissue engineering and regenerative medicine, as well as for drug delivery applications, there is demand for the development of biodegradable materials with broad adjustability of their biochemical and biophysical properties. Furthermore, it is desired that these materials possess the ability to covalently incorporate surface and/or bulk modifications. Previously, we developed a platform of three-armed biodegradable macromers consisting of trivalent alcohols with varying degree of ethoxylation (0 to 7 ethylene glycol units) modified with biodegradable lactic acid (LA) or caprolactone oligoesters. The termination of the arms with methacrylic acid allows for the cross-polymerization of macromers for the generation of solid or macroporous structures [1].



For this work, the basic Tri134(LA6)3.75 (non-ethoxylated trivalent alcohol, 6 LA/arm, full methacrylation with 3.75 eq. methacryloyl chloride during synthesis) was used as reference. To expand the macromer material platform towards a broader adjustability of degradation properties, the degree of macromer methacrylation was (1) reduced to retain arms that terminate with non-methacrylated hydroxyl groups. (2) Furthermore, glycolide was copolymerized with lactide during synthesis to yield oligoester arms that incorporate lactic as well as the more hydrophilic glycolic acid (GA). Using solid lipid templating in combination with thermally induced cross-polymerization, macromers were processed into macroporous scaffolds, which displayed a porosity of around 80%. The fabricated scaffolds were assessed for their hydrolytic *in vitro* degradation behaviour by monitoring mass and mechanical stability.
For scaffolds made from Tri134(LA6)3.75, onset of degradation occurred after 4 months of incubation, and loss of more than 50% of the initial mass was observed after between 9 and 11 months of degradation. Modification of the degree of methacrylation via reduction of the amount of methacryloyl chloride during synthesis resulted in a reduction of the onset of degradation to around 3 to 3.5 months and a loss of more than 50% of initial mass after 5.5 to 6.5 months, but did not significantly affect initial compressive modulus of the scaffolds (25-30 MPa). Incorporation of a low amount of GA (Tri134(LA5GA1)3.75) similarly did not affect initial compressive modulus, but resulted in an even earlier onset of degradation after about 2 months of incubation and a pronounced acceleration of degradation, with loss of 50% of initial mass after about 4

POS.171

Functionalization of copolymerized films from three-armed biodegradable macromers

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For guided tissue regeneration, surface properties of bone substitution materials are of high relevance. Our approach is to modify 3-armed methacrylated oligolactid-based macromer matrices[1] that have been shown to improve bone defect healing in diabetic rats[2]. By using an established protocol to generate macromere-derived polymer films, we are able to analyse the surface properties of equally composed scaffolds[3].
In a new attempt, we decided to incorporate a small molecule anchor into the polymer network, which then binds a suitable linker. This way, we are able to vary the solvents used during polymerisation and add further modifications to these surfaces to achieve the desired effects. Surface decoration with sulfated glycosaminoglycans (GAG) are intended to improve bone defect regeneration.
We engineered surfaces from a toolbox of biodegradable macromers that can be cross-copolymerised with glycidyl methacrylate. A further incubation with polyetheramines (Jeffamine®) results in amino-functionalised films, which then are available for further functionalisation. By testing different polymerisation and incubation conditions, we were able to establish a reproducible protocol for the above-mentioned process.
The amount of amino-groups available has been shown by staining the surfaces with 5-(6)-SFX as fluorescent dye.
By narrowing down the used binary solvent to a one-solvent system we were able to avoid unwanted effects e.g. phase separations and received a high and evenly spread surface presentation of glycidyl methacrylate and thereby amino groups on the surface of the films.
In a next step we immobilised sulfated GAGs on these surfaces by using EDC in combination with NHS as an carbodiimide crosslinker. Due to their fluorescent labelling we were able to detect and quantify the amount of GAGs available. In order to differentiate between covalently and adsorptively immobilised sulfated GAGs we established a washing protocol using a KSCN buffer.
After successfully doing this, first scavenging studies for the bone catabolic protein sclerostin were performed.
During these experiments, we were able to show a significant scavenging effect of the sGAG modified surfaces compared to the control group.

In the future, these materials & methods should be transferred to 3-D scaffolds to improve bone regeneration via scavenging of bone catabolic proteins.

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POS.172

Multivalent block-co-polymer nanoparticles bind to target cells with high avidity

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Block-co-polymers provide a tremendous toolbox for the preparation of biodegradable nanoparticles (NPs) for biomedical applications. It is straightforward to design and manufacture NPs with different size, surface modification and ligand density for receptor interaction. We designed NPs consisting of poly(ethyleneglycol)-poly(lactic acid) block-co-polymers as well as poly(lactic-co-glycolic acid) and investigated the binding efficacy of losartan carboxylic acid (EXP3174) carrying NPs (EXP-NP) to angiotensin-II-
receptor type 1 (AT1R) positive cells via their silencing effect on AT1R downstream signalling. Since AT1R is a Gq-coupled GPCR, its activation via the physiological agonist angiotensin II (AngII) leads to an influx of calcium ions into the cytoplasm that can be monitored using calcium-sensitive fluorescent dyes such as Fura2–AM.[1] Pre-incubation of receptor positive cells with increasing EXP-NP concentrations prevents this AngII-mediated calcium influx and allows for the determination of inhibitory concentration constants (IC50) which reflect the avidity of such particles.
Free EXP3174 is able to block AT1R signalling in low nanomolar concentrations (**Figure 1**). As expected covalent coupling of EXP3174 to poly(ethyleneglycol) (EXP-PEG) led to a clear affinity loss. However, EXP-NPs carry a plethora of EXP-PEG arms and allow thus for multivalent effects during AT1R binding. The avidity which may result from this multivalent interaction between NPs and cells is not reflected when calcium concentrations are plotted against ligand concentrations. With respect to the global EXP3174-concentration we observed, in contrast, an additional affinity loss, which is due to NP geometry. Since our particles are spherical, EXP3174 ligands on the backside of membrane bound NPs cannot reach target receptors (**Figure 2**) but contribute massively to the overall ligand concentration in the setup. To overcome this limitation, we calculated the particle number concentration (PNC) from the total polymer mass per sample volume, the particle radius as measured by dynamic light scattering and the particle density taken from the literature.[2-4] Alternatively, we measured PNC values using nanotracking analysis (NTA). The values were in close agreement and provided us with particle-based IC50 values in the sub-nanomolar range reflecting the high avidity of our multivalently binding NPs.

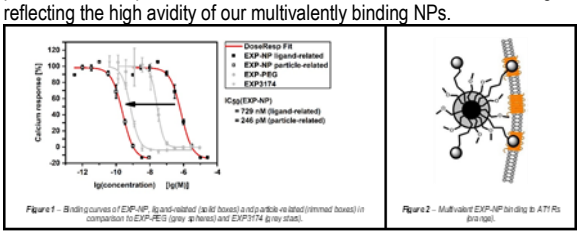

Figure 1 – Binding curves of EXP-NP, ligand-related (solid boxes) and particle-related (rimmed boxes) in comparison to EXP-PEG (grey spheres) and EXP3174 (grey stars).

Figure 2 – Multivalent EXP-NP binding to AT1Rs (orange).

Acknowledgments:

We would like to thank Prof. Karsten Maeder, Department of Pharmaceutical Technology and Biopharmaceutics, Martin-Luther-University Halle-Wittenberg for enabeling the NTA measurements. Financial support from the German Research Foundation (DFG) is gratefully acknowledged (grant number GO565/18-1).

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POS.173

Intended and Unintended Targeting of Polymeric Nanocarriers

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Biodegradable nanoparticles based on stearic acid-modified poly(glycerol adipate) (PGAS) are promising carriers for drug delivery. In order to investigate the impact of the particle interface characteristics on the biological fate, PGAS nanoparticles were covalently and non-covalently coated with N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers. HPMA copolymer-modified PGAS nanoparticles had similar particle sizes, but less negative zeta-potentials. Nanoparticles were double-labeled with the fluorescent dyes DiR (non-covalently) and DYOMICS-676 (covalently bound to HPMA copolymer), and their biodistribution was investigated noninvasively by multispectral optical imaging. Both covalent and non-covalent coatings caused changes in the pharmacokinetics and biodistribution in healthy and tumor-bearing mice [1]. In addition to the intended tumor accumulation, high signals of both fluorescent dyes were also observed in other organs, including liver, ovaries, adrenal glands and bone (Figure 1). The unintended accumulation of nanocarriers needs further detailed and systematic investigations, especially with respect to the observed ovarian and adrenal gland accumulation. The present study confirms previous results of ovarian accumulation of PLGA nanoparticles and lipid nanoemulsions [2] and other lipid based nanodispersions, using different and multiple labelling techniques [3, 4]. Therefore, the ovarian and adrenal accumulation of nanomaterials is not an uncommon phenomenon.

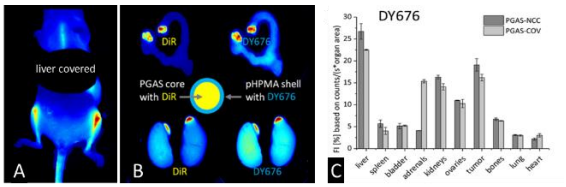

Figure 1: A: In vivo Image (5 min) of uncoated PGAS-nanoparticles showing early accumulation in bones; B: Ex vivo Optical Images of HPMA-coated PGAS nanoparticles sowing accumulation in ovaries and adrenals; C: Ex vivo signal intensity distribution of DY676-labeled HPMA-PGAS nanoparticles.

The authors gratefully acknowledge the support by Deutsche Forschungsgemeinschaft DFG (MA 1648/8-1).

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3.11 Pharmacology

POS.174

In-vivo disintegration and absorption of two fast acting aspirin tablet formulations compared to ibuprofen tablet formulations using combined gamma scintigraphy and pharmacokinetics

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Aspirin (acetylsalicylic acid, ASA) is widely used to relieve acute pain and reduce fever and inflammation. New 500 mg and 1000 mg aspirin rapid disintegrating tablets with small active ingredient particle size and sodium carbonate excipient were developed to replace the regular 500 mg aspirin tablet formulation containing starch and cellulose. The rapid disintegrating aspirin tablets have been proven to show faster in-vitro dissolution and a shorter time to C_{max} (1) resulting in faster pain relief (2). The current study correlates the site and time to complete tablet disintegration with the plasma concentration time profile, using γ-scintigraphy imaging technique. Two 400 mg ibuprofen tablet formulations (ibuprofen acid and ibuprofen lysine salt) have also been analyzed in this study.
The study was open label, randomized, and four-way crossover trial in 12 healthy fasted males. Scintigraphy images and blood sampling were taken up to 4h post dose. Tablets were radiolabelled with 4 MBq technetium (99mTc)-labeled DTPA using a drill and fill method (3,4). Site and time to complete tablet disintegration were derived from the scintigraphy images. Validated bioassays were used to measure plasma concentration.
In all subjects the aspirin formulations disintegrated rapidly and completely in the stomach (500 mg Aspirin: 9.0 min, 1000 mg Aspirin: 5.0 min). Complete disintegration of the ibuprofen tablets occurred in 5 and 4 out of 12 subjects in the small intestine. Time to complete disintegration was longer (acid: 37.5 min, lysine-salt: 37.5 min) than with the aspirin formulations. This correlates with pharmacokinetics: a substantial difference in T_{max} between the two aspirin formulations (500 mg Aspirin: 20 min, 1000 mg Aspirin: 23 min) and the two ibuprofen formulations (acid: 68 min, lysine-salt: 42 min) was observed.
The gastrointestinal disintegration and systemic absorption of the aspirin formulations is faster compared to the ibuprofen formulations.

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POS.175
PST.09

Effect of cell culture on the biphasic kinetics of insulin secretion

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The biphasic kinetics of insulin secretion is indispensable for the maintenance of the glucose homeostasis of the organism as evidenced by its progressive during type2 diabetes. It is widely accepted that the insulin release from perifused isolated islets faithfully represents the kinetics of insulin secretion in vivo. It has become a routine measure to use islets only after a period of cell culture to permit a recovery from the collagenase isolation procedure. Here we show that overnight cell culture in RPMI 1640 medium plus FCS profoundly affects the secretion kinetics in response to the nutrient secretagogues glucose and alpha-ketoisocaproic acid (KIC), but much less so to purely depolarizing stimuli (tolbutamide or high extracellular potassium). The kinetics which is regarded to be the typical response of mouse islets to glucose, namely the strong first phase followed by an elevated plateau as the second phase is visible after the cell culture period, whereas freshly isolated islets show a markedly ascending second phase. This transformation can also be observed with microdissected islets which have not undergone the stress of collagenase digestion. In cultured islets the nutrient (either glucose or alpha-ketoisocaproic acid)-induced increase in NAD(P)H-autofluorescence and decrease in FAD-autofluorescence was more marked than in freshly isolated islets, but the kinetics was unchanged. This could indicate a stronger driving force for ATP-generation and a predominance of the triggering pathway over the amplifying pathway. To assess the role of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) for the culture-dependent change of secretion kinetics the $[Ca^{2+}]_i$ of cultured and of freshly isolated islets was measured simultaneously with the insulin secretion. The $[Ca^{2+}]_i$ pattern in freshly isolated islets in response to 30 mM glucose was similar to that of cultured islets and did not reflect the difference in the secretory pattern. The role of the main components of the cell culture medium, RPMI and FCS, in shaping the kinetics of secretion was assessed by replacing RPMI with Krebs-Ringer medium and 10 % (v/v) FCS with 0.4 % (w/v) BSA. The cell culture period also elicited the response pattern with a plateau after a marked first phase when FCS was replaced by BSA and RPMI with Krebs-Ringer, however FCS elevates mainly the secretion level during the second phase more than BSA. It is known that the pre-stimulatory condition of is of major importance for the kinetics of stimulated insulin secretion. In this regard the cell culture period may not so much exert a recovering but rather a standardizing influence on islet function.

POS.176

Ingredients of *Artemisia annua* Dietary Supplements Are Cytotoxic for Highly Metastatic Triple Negative Human Breast Cancer Cells

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Artemisia annua L., a Chinese medicinal plant, is well-known for its antimalarial activity. In recent years, *Artemisia annua* has gained increasing attention for its activity towards other diseases including various types of cancer. In particular, artemisinin and its semisynthetic derivatives became subject of intense investigations on their molecular mode of action and some are currently being tested in clinical phase I and II trials [1].
Available evidence suggests that artemisinin might not be the most active ingredient of *Artemisia annua* [2,3]. The plant contains a multiplicity of other biologically active substances indicating that *Artemisia annua* might be a source of new herbal anticancer therapeutics, which merits further investigation.
Considering the fact that natural products exhibit unique chemical structures selected by evolutionary pressure for interaction with proteins and biological targets [4], we have analyzed different *Artemisia annua* extracts marketed as dietary supplements for their activity against the highly metastatic, triple negative breast cancer cell line MDA-MB-231. The extracts were chemically characterized by reversed phase HPLC/MS/MS and extracts of 3 out of four dietary supplements were found to contain no detectable artemisinin. Nevertheless, such extracts also exhibited antiproliferative and apoptosis inducing effects on the MDA-MB-231 breast cancer cells *in vitro* and *in vivo*. To identify new ingredients with antiproliferative and cytotoxic effects, a bioguided fractionation was conducted. The fractions of the extract with high cytotoxic activity were isolated by semipreparative HPLC and the structures of individual compounds were identified by mass spectrometry and ^{1}H- and ^{13}C-NMR spectroscopy. Extracts as well as the isolated compounds 6,7-dimethoxy-coumarin, chrysosplenol D, casticin,

arteannuic acid and arteannuin B, which had been purified to chemical homogeneity, were analyzed *in vitro* and *in vivo* for potential anticancer activities.
Artemisia annua extract and the individual compounds exhibited time- and concentration-dependent toxicity against several cancer cell lines. Interestingly, extract and arteannuin B, one of the isolated compounds, exhibited selective cytotoxicity against cancer cells, whereas normal mammary epithelial cells remained unaffected at equivalent concentrations demonstrating selectivity towards cancer cells. Moreover, treatment with an *Artemisia annua* extract or with the individual compounds inhibited cell proliferation by affecting the cell-cycle progression. The cancer cells accumulated in the S-phase after treatment with the extract, whereas S- and G_2/M-phase arrest was observed after treatment with the isolated compounds. The treated cancer cells exhibited apoptotic cell death, which was identified by flow cytometry demonstrating DNA-fragmentation, activation of caspase 3/7 and annexin V/propidium iodide staining. Loss of the mitochondrial membrane potential suggests involvement of the intrinsic apoptotic pathway. Furthermore, antiproliferative and apoptosis-inducing efficacies were confirmed *in vivo* using a 3D breast cancer xenografts grown on the chorioallantoic membrane of fertilized chick embryos, where the *Artemisia annua* extract and the isolated substances significantly reduced the MDA-MB-231 breast cancer tumor volume. Likewise, the breast cancer xenografts exhibited reduced expression of luciferase and, hence, reduced bioluminescence as detected by IVIS, as well as a lower expression of the immunohistochemical proliferation marker Ki-67. Importantly, no systemic toxic effects of the extract or the individual compounds on the chick embryos could be detected.
This study enables new insights into the rational use of *Artemisia annua*-derived compounds in anticancer therapy and has identified compounds beside artemisinin with potent anticancer activity against a highly metastatic, triple negative human breast cancer cell line. This study supports the notion that dietary supplements may contain therapeutically active compounds.

Acknowledgements: This work was supported by Akademisches Zentrum Komplementäre und Integrative Medizin (AZKIM), funded by the Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg

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POS.177

Role of Cysteine-rich LIM-only protein 4 (CRP4) for molecular and cellular mechanisms of vascular remodelling

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Background:
LIM domain proteins have been identified as essential proteins for normal cardiac development and function. Studying the hearts of cysteine-rich LIM-only protein 4 (CRP4) deficient mice, we demonstrated that the ablation of CRP4 exaggerates multiple parameters of the cardiac stress response to the neurohormone angiotensin II. The loss of cardioprotective CRP4 was partly compensated by an upregulation of CRIP1 (cysteine-rich intestinal protein 1), another LIM-protein highly homologous to CRP4 [1]. LIM-domains are generally considered as protein binding interfaces and serve as adapter proteins as well as transcription factors [2]. However, the function of these LIM proteins in the vasculature in health and disease remain largely elusive. So far, it has been shown, that vascular CRP4 is a new substrate of the cGMP-dependent protein kinase I (cGKI) and acts synergistically with the transcription factors GATA-6 and serum response factor (SRF) to control the expression of smooth muscle specific genes [3,4]. Since dysfunctions of vascular smooth muscle cells (VSMC), particularly the phenotypic switch of a differentiated and contractile to a synthetic and dedifferentiated phenotype are indicative for many vascular diseases such as hypertension or atherosclerosis, we are aiming to investigate the impact of CRP4 for the modulation of VSMCs.

Methods:
The molecular characterization of VSMCs was evaluated using mice with a deletion of CRP4 (CRP4-KO) and their littermate controls (CRP4-WT) by dissecting the aorta and applying an *in vitro* cell culture system of VSMCs. The subcellular localization of CRP4 as well as typical smooth muscle proteins were studied by immunohistochemistry, immunofluorescence and separation of subcellular fractions. Since alterations in proliferation and cell migration are hallmarks of modulated VSMCs, we studied the CRP4-dependent migratory behaviour of cultivated VSMCs by a modified scratch assay at baseline and in response to different stimuli such as platelet-derived growth factor BB (PDGF-BB) and cyclic guanosine-3',5'-monophosphate (cGMP)-analogons. The CRP4-dependent proliferation of VSMCs was carried out by impedance based measurements using the xCELLigence system as well as Ki-67 staining and cell counting by using cell culture plates with a grid. Changes of typical marker genes and proteins of contractile or synthetic VSMCs to evaluate the CRP4-dependent modulation of the vasculature were assessed by quantitative real-time PCR and Western Blotting respectively.

Results and conclusion:
In VSMCs, CRP4 is localized in the cytoplasm, especially in the perinuclear region, whereas its homologue CRIP1, is particularly expressed in the nucleus of VSMCs, which points to different functions of these LIM-proteins in the vasculature. Yet distinct changes regarding the expression profile of typical smooth muscle genes and proteins in contractile versus synthetic smooth muscle cells became apparent by comparing cultivated VSMCs of CRP4-WT and CRP4-KO animals. These results are congruent to an observed CRP4-dependency regarding the onset of the shape change of cultured VSMCs. Moreover, the present findings suggest an important role of CRP4 for the behaviour of VSMCs, which provides evidence to a distinct proliferative and migratory phenotype. Therefore, CRP4 may represent a crucial factor for the onset and pathogenesis of vascular diseases and must be further investigated by *in vivo* approaches using mouse models of elevated blood pressure, atherosclerosis and aneurysms.

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POS.178

Enantiomeric Discrimination in Phase-I Metabolism on Propranolol Monitored by SFC-MS/MS

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Chiral recognition of drugs and their metabolites plays an important role for comprehension of pharmacodynamic effects and pharmacokinetic behaviour of chiral biologically active compounds. Published by the FDA and EMA in 1992 and 1993, respectively, guidelines shall ensure to consider each enantiomer of pharmaceuticals as single active compound. Chiral SFC techniques may offer an opportunity for fast enantiomeric discrimination of diverse types of analytes within minutes or even seconds in biological analysis [1]. Highly sensitive mass

detectors hyphenated to SFC enable the detection of trace amounts of drugs and metabolites in doping control, forensic investigations and therapeutic drug monitoring. High efficiency, short analysis time and low organic solvent consumption facilitate application as high throughput routine methods.
The β-blocker propranolol undergoes extensive phase-1 and phase-2 metabolism. In literature it is reported that CYP2D6, CYP1A2, CYP2C19 are involved in catalysing ring oxidation, side chain dealkylation and further side chain oxidation of propranolol [2]. Furthermore, it is reported that R-propranolol is preferred in human phase-1 metabolism and consequently exhibits a shorter half-life [3]. Thus, S-propranolol is about 100-times more active than R-propranolol. The ring hydroxylated propranolol metabolite 4'-hydroxypropranolol also possesses pharmaceutical activity.
Within this study an Agilent 1260 Supercritical Fluid Chromatography System coupled to an Agilent Triple Quadrupole 6495 was used. As chiral stationary phase tris-(3,5-dimethylphenyl) carbamylated cellulose, namely Chiralpak IB (5 µm, 4.6 mm x 250 mm), was utilized.
4'-, 5'-, and 7'-Hydroxy metabolites, N-desisopropylpropranolol and 4'-hydroxypropranololsulfate were chemically synthesized as reference substances. For proof of concept, chiral discrimination of generated phase-1 metabolites was analyzed in biological samples.
Enantioseparation of the parent drug propranolol, 4'-hydroxypropranolol, 5'-hydroxypropranolol, 7'-hydroxypropranolol, N-Desisopropylpropranolol and 4'-hydroxypropranololsulfate is investigated and detailed SFC-system settings and method parameters are presented and discussed.

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POS.179

In-Vitro Investigations on the Effect of NSAID's on Human Steroid Metabolism

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Introduction: To detect misuse of pseudo endogenous steroids as doping substances, anti-doping laboratories monitor urinary concentrations and ratios of various endogenously produced steroid hormones, their precursors, and metabolites. However, recently it was shown that some (non-prohibited) drugs may influence the metabolism of urinary excreted steroids [1]. Due to the complexity of the human steroid metabolism (see Figure 1), several other drugs may also influence the urinary steroid profile by interfering with steroid metabolizing enzymes.

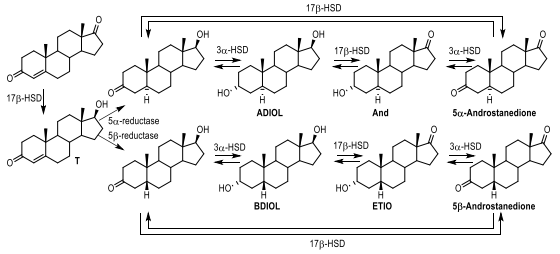

Figure 1: Testosterone metabolism focusing on steroid profile ratios detected in anti-doping analysis

Objective: Non-steroidal anti-inflammatory drugs (NSAIDs), as most frequently used drugs in sports, are an example for those potentially interfering substances. NSAIDs have been shown to inhibit steroid metabolizing aldo-keto-reductases (AKR) [2]. Ibuprofen was reported as inhibitor of AKR1C2 [2]. As AKR1C2 catalyses formation of Androsterone (And) from 5α-Androstanedione, an inhibition may result in a decreased And/Testosterone (T) ratio.
Indomethacin was found to selectively inhibit AKR1C3 and thus conversion of Androst-4-ene-3,17-dione (AED) to T [2]. Hence it is expected, that the T/EpiT ratio in the urinary steroid profile may be reduced.
As no profound information on the influence of NSAID intake on human steroid profile is available in literature, this work aims to close this gap.
Methods: Incubations with recombinant human AKR1C enzymes were performed. Formation of products was monitored by GC/MS measurements. Furthermore, kinetic characterisation of steroid-reactions catalysed by AKR1C enzymes and their inhibition through NSAIDs were conducted by monitoring Co-factor (NADPH) concentration.
Results: Product formation in both AKR1C-catalyzed directions was shown for different endogenous steroids. Furthermore, determination of K_m, V_{max} and k_{cat} was performed. Finally, the extend of inhibition by representative NSAIDs was investigated.
Conclusion: The results of this work help to estimate the influence of NSAID-use on the urinary human steroid profile. Urinary excretion studies will be performed shortly to demonstrate the relevance in vivo.

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POS.180

PST.10

The inhibition of c-met in gastric cancer cells leads to the upregulation of HER3 via SATB1

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1. Introduction
The receptor tyrosine kinase (RTK) c-met is amplified in a subset of gastric cancer cells and inhibitors of this RTK exhibit marked antiproliferative effects in these cells. However, owing to their redundant function the acitvation of receptors of the HER family could represent a potential mechanism of resistance against c-met inhibition.
2. Objectives
The aim of the present study was to further delineate the role of HER receptors, especially HER3, in mediating resistance of gastric cancer cells against c-met inhibition.
3. Materials and methods
We used two gastric cancer cell lines with an amplification of c-met (MKN45 and SNU5) and two cell lines without c-met amplification (MKN7 and MKN28) and evaluated the antiproliferative effects (WST-1 assay, growth of tumor spheroids) of pharmacological or siRNA-mediated c-met inhibition as well as the impact of heregulin treatment. Furthermore, the regulation of HER expression after inhibitor treatment was analyzed on mRNA (RT-qPCR) and protein (immunoblot, FACS analyses) level.
4. Results
The siRNA-promoted knockdown of c-met or the use of c-met kinase inhibitors had a pronounced antiproliferative effect in the c-met amplified cell lines but not in cells without c-met amplification. Moreover, treatment of c-met amplified gastric cancer cells with the HER3 agonist beta-heregulin restored the activity of p44/42 MAPK and Akt and partially reversed the antiproliferative effects of c-met inhibitors. Of note, only in the c-met amplified cell lines, treatment with c-met inhibitors or c-met directed siRNA led to a markedly upregulation of HER3 on mRNA and protein level. To further delineate the mechanism of HER3 upregulation upon c-met inhibition, we investigated the role of the transcriptional regulator protein SATB1, which in other tumor entities is known to be involved in the expression of HER receptors. In fact, downregulation of

SATB1 using siRNA abrogated the HER3 induction upon c-met inhibition and impaired the rescue effect of heregulin.
5. Conclusion
Upon c-met inhibition, SATB1 is involved in the compensatory upregulation of HER3. Thus, targeting SATB1 may help to overcome resistance against c-met inhibition in gastric cancer.

POS.181

Anti-inflammatory and cytoprotective effects of *Hypericum perforatum* L. extract

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Introduction: Glutamate toxicity and inflammation are involved in a variety of disorders, e. g. those involving adaptation to chronic stress. In this context, the combined antioxidant and anti-inflammatory properties [1] of St. John's wort (*Hypericum perforatum* L.) extract could contribute to the antidepressant effects [2] by normalization of an overactive hypothalamic-pituitary-adrenal axis [3].

Aims: Thus, the aim of our investigations was to determine the effects of a St. John´s wort extract with clinically proven efficacy in mild to moderate psychic depression [3], STW 3-VI, on protection of differentiated mouse hippocampal HT22 cells from the cytotoxic effects of glutamate or NMDA and the possible anti-inflammatory properties on LPS-activated macrophages (MΦ).

Methods: Differentiated HT22 cells were pre-treated with STW 3-VI to investigate the protective effects against glutamate or NMDA cytotoxicity. The anti-inflammatory properties of STW 3-VI were evaluated by quantification of the TNF release on LPS activated PMA-differentiated THP 1 MΦ using ELISA assay and the mRNA expression of TNF and IL-6 by qRT-PCR. Glutamate or NMDA (0.1mM) induced 30% cytotoxicity in HT22 cells.

Results: Pre-incubation (24h) of STW 3-VI improved the viability by 30%, compared to the control. Pre-treatment (48h) of LPS-activated MΦ with STW 3-VI induced a significant lowering (54%, 64% and 53%) of TNF release. QRT-PCR revealed that 48 h pre-treatment with STW 3-VI inhibits the mRNA expression of IL-6 and TNF respectively by LPS-activated MΦ.

Conclusion: STW 3-VI protects hippocampal cells from glutamate or NMDA induced cytotoxicity and activates the anti-inflammatory defense by inhibition of the cytokine production by MΦ, These effects might be relevant for the therapeutic effects of STW 3-VI in psychic depression.

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POS.182

Herbal medicinal products in irritable bowel syndrome: Mechanisms of action of STW 5

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Herbal treatment options are increasingly used in functional gastroenterological disorders (FGID) like irritable bowel syndrome (IBS), while an understanding of their mechanisms of action is often lacking. According to recent national and international therapeutic guidelines (1,2,3), STW 5, a combination product of nine herbal extracts, is an evidence-based treatment option for IBS. This poses the question as to what the mechanisms of action and the utility of its therapeutic efficacy are.
To warrant completeness, a systematic search according to the PRISMA statement was conducted in order to retrieve all data on STW 5 or its trade name (Iberogast), using PubMed, Toxlit and BIOSIS. Identification of data on the mechanisms of action was then done manually. In addition, hand searching was done and text books were screened to get a complete picture.
The search identified 468 publications. A considerable number of publications on spasmolytic as well as prokinetic activities could be identified, as well as on prosecretory effects. Furthermore, data showing that the product can counteract inflammatory changes as well as an intestinal hypersensitivity and hyperpermeability were also found. Even a beneficial effect on the microbiota was described. Accordingly, the product has a multitude of mechanisms of action.
In IBS, a number of therapeutic options with different mechanisms of action are used. A search for the mechanisms of action of a herbal treatment used in this indication (STW 5) revealed not just one, but a multitude of mechanisms of action. This confirms for this product, that its action in IBS can be classified as multi-target and makes its therapeutic efficacy in this indication plausible.

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POS.183

Mode of action of an herbal combination used in painful complaints in degenerative and inflammatory rheumatic diseases

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Herbal medicinal products such as STW 1, a combination product containing extracts of trembling poplar leaves and bark, golden rod herb and common ash bark, are used in painful complaints caused by degenerative and inflammatory rheumatic diseases. This use is based on a large number of clinical studies [1]. Therefore, the question is which mechanisms of action contribute to this therapeutic effect?
Accordingly, a review of all published data was conducted with the aim to identify data on the pharmacology of this product.
As could be shown, the actions of the combination product as well as those of its components are based on antiinflammatory, antioedematous, antioxidative and analgesic properties. These modes of action are even broader than those of synthetic antirheumatics.
Accordingly, these data can explain the action and efficacy of STW 1 in randomised, placebo- or verum-controlled double-blind trials, as well as in non-interventional studies, which have been performed in different subtypes of rheumatic diseases and even documented as a successful combination therapy with non-steroidal antiinflammatory drugs (NSAIDs).
Accordingly, STW 1 complements NSAIDs and cyclooxygenase (COX-2) inhibitors in particular in these indications.

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POS.184

Synergy – a key to the multi-target action of natural products

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Introduction: Natural products are typically characterized by their multitude of constituents addressing multiple targets in the organism. Their action has therefore been often postulated to be based on a multi drug/multi target action [1]. This has been successfully proven for an herbal medicinal extract combination used in functional gastrointestinal diseases, STW 5 [2], as its action is based on very different effects in different regions of the stomach and the intestine [3]. With a multiplicity of targets being the basis of its proven therapeutic efficacy and safety, synergy is assumed to be a key for its action.
Methods: As a model, rat and guinea pig small intestinal smooth muscle preparations, stimulated with ACh [4], or incubated with TNBS for inducing an inflammation [5], were used. STW 5 and its components, alone or in combinations, were tested. A Box-Behnken-Design and the isobologram method were used for analysis.
Results: The smooth muscle-relaxing effects of STW 5 were supra-additive in the model of ACh-induced contraction in comparison to the single components. In the TNBS-model, synergistic, additive as well as antagonistic effects were identified, depending from the combinations of extracts tested.
Discussion: Our results support the concept of a multi-target therapy, with synergy as a key factor. Further evidence for this concept is presently generated by modern gene expression profiling methods [6, 7] giving a rationale for the good clinical efficacy and safety of natural products used in modern phytotherapy, and proven in modern clinical trials.

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POS.185

Action of aqueous willow bark extract in rheumatic complaints, fever and headache - more than just tradition

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Willow bark extracts are authorized for rheumatic complaints and headache, and are recommended in therapeutic guidelines of AWMF, the . As they contain a large number of polar constituents, the salicylates being the basis of their standardization, relevance of these for the mode of action is a matter of interest, especially with regard to the use of aqueous willow bark extracts as STW 33-I in therapy.
Therefore a literature review was conducted, focused on the mode of action of willow bark extracts and fractions thereof.
This review revealed a considerable number of pharmacological studies [1], including in vitro- and in vivo studies in different pharmacological models [2], supporting the assumption that especially the polar fractions of the extract contribute to the anti-inflammatory action. Salicylic acid derivatives and their metabolites [3], together with different other polyphenols, were identified as relevant for the mechanisms of action, as was also confirmed by gene expression analyses [4].
Accordingly, by studies on the mode of action of an aqueous willow bark extract, STW 33-I, it could be shown, that the action is based on more than just tradition, given on the broad available evidence on willow bark and the salicylates, and can be well explained on a molecular level.

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POS.186

Effects of Hypericum perforatum extract, miquelianin and hyperforin on CRH induced HPA axis activation in pituitary-derived AtT-20 cells

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Introduction: Normalization of the often hyperactive HPA system in depressive patients occurs during successful antidepressant pharmacotherapy [1]. FK506 binding protein 51 (FKBP5) plays a crucial role in HPA axis functioning. In a recent RT-PCR study [2], we found remarkable effects on FKBP5 expression after dexamethasone induced stress in SH-SY5Y cells. A St. John's wort extract (STW3-VI) as well as miquelianin and hyperforin could reverse the glucocorticoid induced increase in FKBP5 mRNA expression.
These results lead us to the question whether similar effects might be seen in non-neuronal HPA-axis relevant cells. The pituitary adenoma cell line AtT-20 was used to demonstrate HPA axis response to CRH induced stress. Key components of the HPA axis were investigated under stress conditions after treatment with STW3-VI, hyperforin, miquelianin and citalopram. In addition, it was examined whether cytokines are involved in the CRH-induced increase in HPA-axis related gene expression.
Materials and Methods: AtT20 cells were treated with CRH to mimick in vitro stress conditions. Real-time PCR and immunofluorescence experiments were conducted to assess the gene expression and protein expression of POMC, FKBP5 and the cytokines IL-1β and IL-6 under the various treatment conditions.
Results and Discussion: FKBP5 and IL-6 were upregulated after 24h of CRH treatment (1.6 and 1.4 fold, respectively). CRH treatment for 24h did not significantly increase pro-opiomelanocortin (POMC) expression in AtT20 cells (1.2 fold). Co-incubation with citalopram, miquelianin and hyperforin for 24h reduced the CRH-stress induced mRNA increase of FKBP5. CRH stress had a pronounced effect on IL-1β expression which led to an approx. 132 fold increase. This marked increase in IL-1β mRNA expression was completely reversed by STW3-VI, miquelianin and citalopram,
Conclusions: Our present findings support the hypothesis [3] that the anti-inflammatory properties of St. John's wort extract and some of its compounds could contribute to the antidepressant effects through normalization of an overactive HPA axis.

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POS.187

Clinical implications of incomplete complement inhibition by the therapeutic antibody Eculizumab (Soliris®): experimental and clinical data

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Introduction: Eculizumab (Soliris®), a monoclonal antibody directed against the complement protein C5, is a first-in-class biologic drug that inhibits the terminal and lytic pathway of complement. It is approved for clinical use in three rare but very serious diseases: the neurological condition of generalized anti-acetylcholine receptor (AchR) antibody-positive myasthenia gravis (gMG), the kidney disease atypical haemolytic syndrome (aHUS) and the haematological disease paroxysmal nocturnal haemoglobinuria (PNH). While all three diseases are associated with developing various disabilities, the latter two are also characterised by a high mortality rate if not treated with Soliris®. Therapy with Eculizumab brought tremendous clinical benefits for the patients: the high mortality for aHUS or PNH patients was reduced to that of the normal age-matched population. The potential of anti-complement therapeutics in the future is further corroborated by 18 active clinical trials which investigate the efficacy of Eculizumab in other indications like e.g. "prevention and treatment of kidney graft reperfusion injury". However, apart from the dramatic benefits Eculizumab therapy has introduced, there are also disadvantages: (i) few people with a rare polymorphism in the C5 gene do not respond at all to Eculizumab; (ii) about one third of PNH patients respond incompletely and remain transfusion dependent; (iii) with costs of about $ 400 000 per patient per year Eculizumab is one of the most expensive therapies available in the clinic.
Contrary to dogma, we have shown previously that Eculizumab, which binds the human complement protein C5 with picomolar affinity, does not always inhibit C5 completely resulting in residual C5 activity and thus residual terminal complement pathway activity[1,2]. We proved mechanistically that strong complement activation overrides the C5 inhibition by Eculizumab or other stoichiometric C5 inhibitors and demonstrated that residual C5 activity can be observed ex vivo in serum form PNH patients despite of excess amounts of Eculizumab.
Objectives: Here we investigate if the ex vivo levels of residual C5 activity in patient sera differ across different PNH patients on Eculizumab treatment and whether any differences in residual lytic activity correlate with the clinical responses. We also investigate if clinical signs of strong complement activation in vivo coincide with intravascular hemolysis and thus impair the clinical response to Eculizumab.
The underlying hypotheses are that especially high levels of residual C5 activity in sera of Eculizumab treated patients are associated with severe clinical symptoms and thus impair the therapeutic benefits.
Results: Analysis of 15 PNH patient samples showed that the level of residual C5 activity in an analytical ex vivo assay on rabbit erythrocytes differs markedly with a factor of up to 3.4 among different patients (range of 11.0 – 37.4 %). Since Eculizumab inhibits the complement terminal pathway, hemolysis of PNH erythrocytes, which is a hallmark of the disease PNH, is usually inhibited under Eculizumab therapy resulting in a marked reduction of the high lactate dehydrogenase (LDH) levels in treated patients. We plotted the clinical LDH levels of all patients in this study against the levels of residual hemolysis determined in the ex vivo assay and found that the patient with the highest residual C5 activity of 37.4% continuously exhibits dramatically (~twofold) increased LDH levels and clinical symptoms despite of this patient being administered higher than usual doses of the drug. For the other patients' samples residual hemolysis levels ranged from 11-30% and no long term abnormality in LDH levels is evident. However, some of these latter patients exhibit dramatic, but only punctually increased LDH levels for very short periods. For seven patients with sporadic, short-term peaks in LDH proof of infections at the time points of hemolysis was found. Thus, clinical manifestations can also occur for patients with the commonly observed moderate levels of residual terminal pathway activity under Eculizumab: in cases of infections that strongly activate complement the residual C5 activity exacerbates and causes lysis of PNH erythrocytes alongside clinical symptoms.
Conclusions: This study provides evidence that ongoing high levels of residual C5 activity in a PNH patient receiving Eculizumab impairs the clinical response as measured by patient's LDH levels and that commonly observed levels of residual complement C5 activity exacerbates during infections and causes "pharmacodynamic breakthrough" hemolysis in Eculizumab treated PNH patients.

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POS.188

Crtc1-deficient mice show reduced induction of fibrosis

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Introduction: Cardiac hypertrophy is a prominent risk factor for heart failure. The cAMP regulated transcriptional coactivator 1 (CRTC1) is regulated by cAMP and calcineurin, which contribute to cardiac hypertrophy. Our previous data have shown an increase in CRTC1 protein in human and murine hearts in pathological hypertrophy. Global *Crtc1*-deficient mice develop spontaneous hypertrophy and reduced systolic function. In the present study, the fibrotic phenotype in *Crtc1*-deficient mice and regulators of fibrosis possibly controlled by *Crtc1* were assessed.

Methods: We performed analysis of collagen staining, gene expression of Connective tissue growth factor (*Ctgf*), Collagen 1α1 (*Col1a1*), and 3α1 (*Col3a1*), and matrix-metalloproteinase (*MMP*) activity by zymography in *Crtc1*-deficient and wild-type hearts. To assess the effect of β-adrenergic induced hypertrophy on mRNA expression, RNA-sequencing of hearts from wild-type mice treated with isoprenaline (30 mg/kg BW per day) for 7 days was performed. Fibrosis regulating genes with differential expression due to isoprenaline treatment were verified in *Crtc1*-deficient hearts by RT-qPCR. Chromatin immunoprecipitation was used to confirm the recruitment of CRTC1 to identified gene promoters. Hearts from *Crtc1*-deficient and wild-type mice treated with isoprenaline (30 mg/kg BW per day) for 14 days were analysed regarding mRNA expression of *Col1a1*, *Col3a1*, and *Ctgf*, and protein expression of tissue inhibitor of metalloproteinases (TIMP) 1, 2, 3, and 4.

Results and conclusion: Hearts of *Crtc1*-deficient mice displayed hypertrophy compared to wild-type mice, but no fibrosis. Collagen histology, as well as *Ctgf*, *Col1a1*, and *Col3a1* mRNA expression did not differ between *Crtc1*-deficient and wild-type hearts (n=9). RNA-sequencing of isoprenaline treated WT hearts revealed an increase in *Timp4* expression (n=5). *Timp4* expression was 47% lower in *Crtc1*-deficient mice than in wild-type (n=9), suggesting that *Crtc1* might regulate fibrosis via *Timp4*. TIMP inhibits MMPs which degrade collagen. Expression of the *Timp* isoforms 1, 2, and 3; and of *Mmp-2* and -9 was unchanged (n=13), but MMP activity was increased 1.7-fold in *Crtc1*-deficient hearts (n=5). Indeed, endogenous CRTC1 was recruited to the *Timp4* promoter in wild-type hearts as revealed by chromatin immunoprecipitation.
Treatment of *Crtc1*-deficient and wild-type mice with isoprenaline for 14 days increased *Col1a1, Col3a1* and *Ctgf* mRNA expression 2.9-fold, 2.7-fold, and 1.9-fold (n=3-6), respectively, in wild-type but not in *Crtc1*-deficient hearts. Protein analysis of TIMP 1, 2, 3, and 4 revealed no difference between *Crtc1*-deficient and wild-type hearts. Further analysis of mRNA expression, histological analysis, and MMP activity will provide further evidence for the relation between *Crtc1*, *Timp4*, and fibrosis.
Our data suggest that a lack of CRTC1 protects from cardiac fibrosis under hypertrophic conditions via reduction of *Timp4*.

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The Crtc1-deficient mice were a kind gift by Jean-René Cardinaux, Center for Psychiatric Neuroscience, Prilly-Lausanne, Switzerland

POS.189

Activation of the dual leucine zipper kinase (DLK) by inhibition of the phosphatase calcineurin

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The structurally distinct immunosuppressive drugs tacrolimus (FK506) and cyclosporine (CsA) decrease insulin gene transcription and induce β-cell apoptosis by inhibiting the phosphatase calcineurin (CN) leading to post-transplant diabetes mellitus [1, 2, 3]. In addition, both drugs and hydrogen peroxide as reactive oxygen species enhance the activity of the dual leucine zipper kinase (DLK) [2, 4, 5]. Our previous data showed that DLK interacts with calcineurin via the putative calcineurin interaction domain LxVP. Mutation of V364 to A (LxAP) resulted in a disruption of DLK-CN interaction thereby increasing DLK catalytic activity and its damaging effect on β-cells. Furthermore, a LxVP-peptide interacts with those amino acids of calcineurin, which are involved in its inhibition by CsA and FK506 [6]. In the present study, the direct regulation of DLK by calcineurin was investigated. Additionally, the effect of high glucose on primary murine islets of β-cell specific DLK deficient mice (β-DLK$^{-/-}$) was examined.

In JEG cells the stimulated CRE-dependent gene transcription was inhibited by CsA and FK506. This inhibition was more pronounced by overexpressed DLK wt and the V364A mutant. Treatment with the calcineurin inhibitors showed an additional inhibitory effect in the DLK V364A mutant transfected cells. In HIT cells only overexpressed DLK wt and its catalytically inactive mutant but not the V364A mutant decreased the calcineurin-dependent nuclear localization of the transcription factor NFAT. In a protein-protein interaction assay with recombinant produced 6x-His tagged full-length calcineurin and MBP (maltose binding protein) fused DLK the amino acids W352 in CNA and V120 in CNB were shown to mediate DLK-CN interaction. Furthermore, the glucose-induced toxicity on islets of Langerhans was attenuated in islets of β-cell specific DLK deficient mice.

These findings show that calcineurin interacts with DLK via amino acids involved in its inhibition by cyclosporine and tacrolimus. In addition, disruption of the DLK-calcineurin interaction increased nuclear translocation of NFAT suggesting that DLK inhibits calcineurin. Furthermore, the competitive displacement of DLK by CsA and FK506 from its calcineurin interaction region might contribute to the increase in DLK kinase activity shown as an enhanced inhibition of CRE-dependent gene transcription. In addition, β-cell specific deletion of DLK protected murine islets from glucotoxicity. Taken together with our previous data, these results suggest that activation of DLK by inhibition of calcineurin via reactive oxygen species and specific calcineurin inhibitors contributes to the loss of β-cell mass and function and ultimately to diabetes.

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